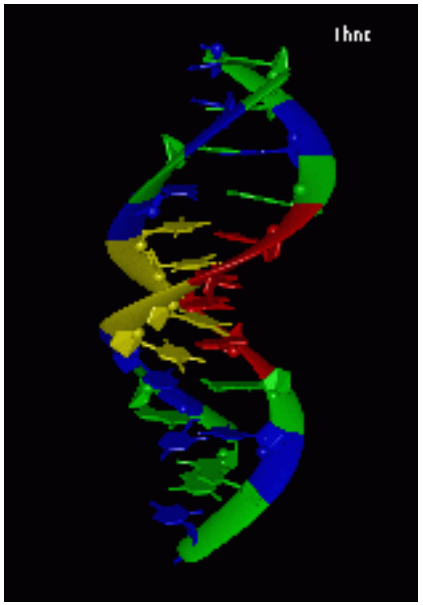
KFC/STBI
Structural Bioinformatics

04_how to get structures experimentally

Karel Berka

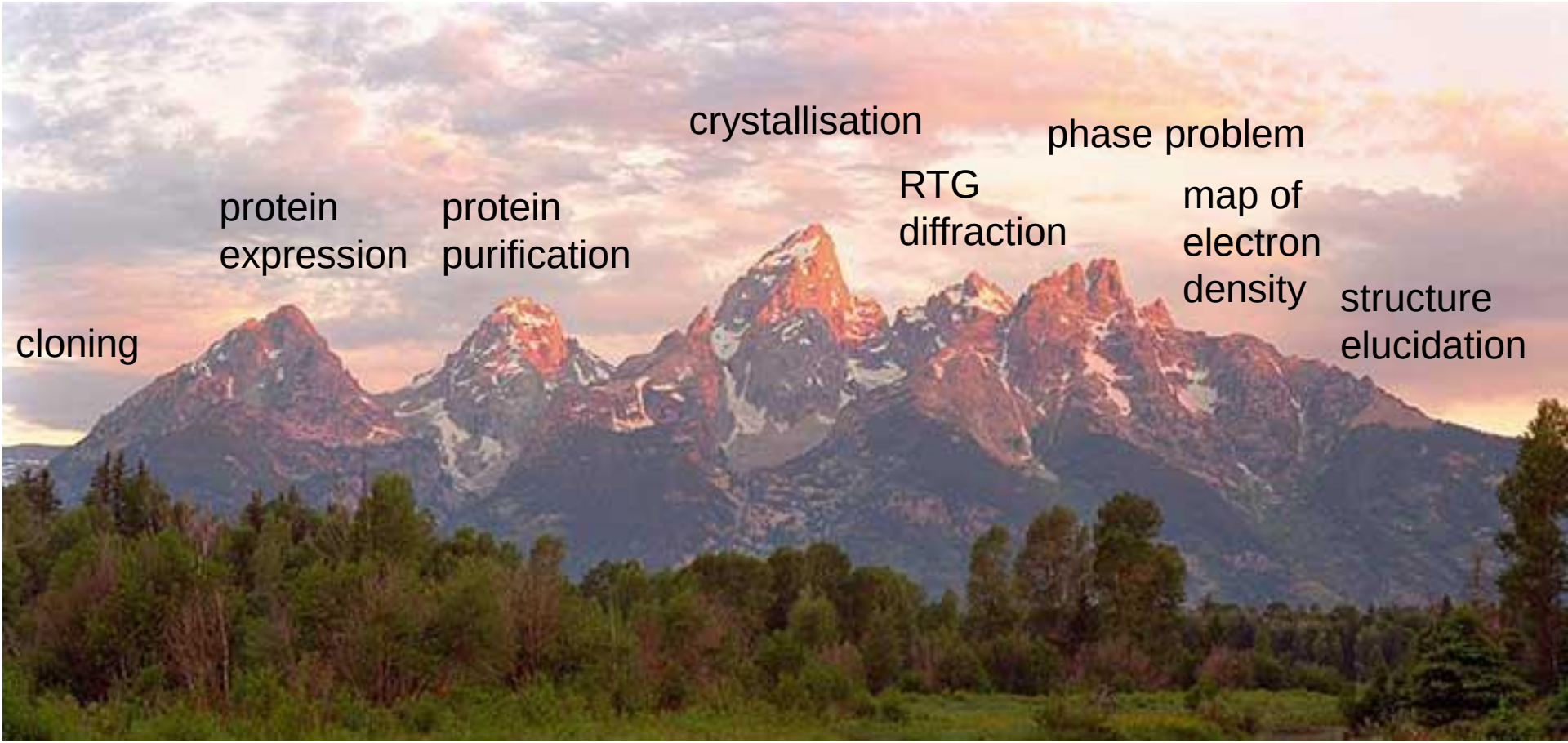
How to get Structure of Macromolecules

- RTG
  - xyz coordinates
  - inner electron shells
  - crystalization, atomic resolution,
  - interpretation of intermolecular interactions
- EM
  - electron shell
  - low resolution
  - large complexes

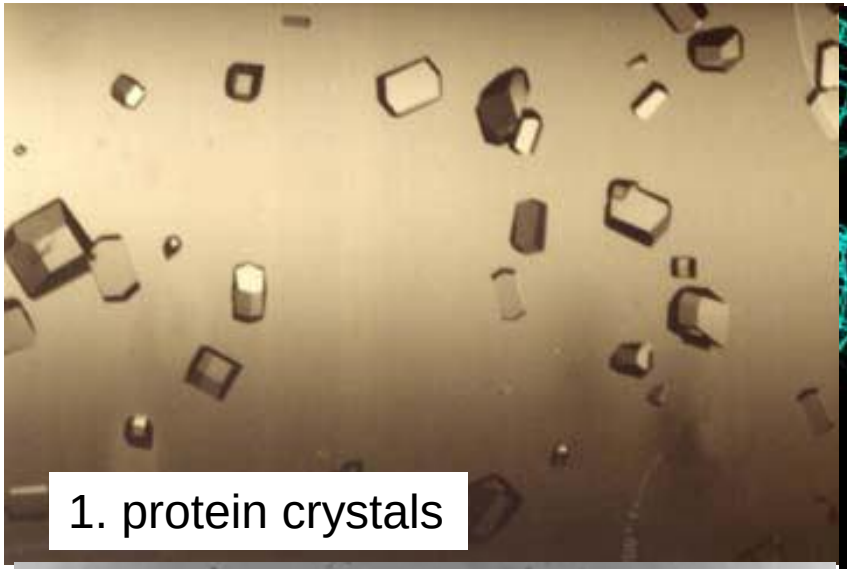


- NMR
  - torsion angles and distances
  - dynamical information available
  - MD model
- MS
  - distances
  - molecular weights
  - solvent accesibility
- and others…
  - FRET - distances

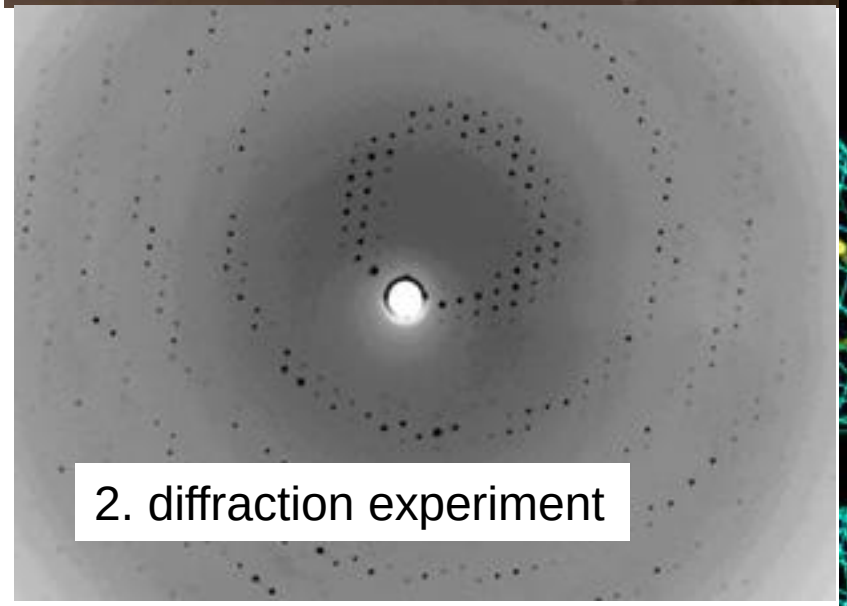
X-ray crystallography



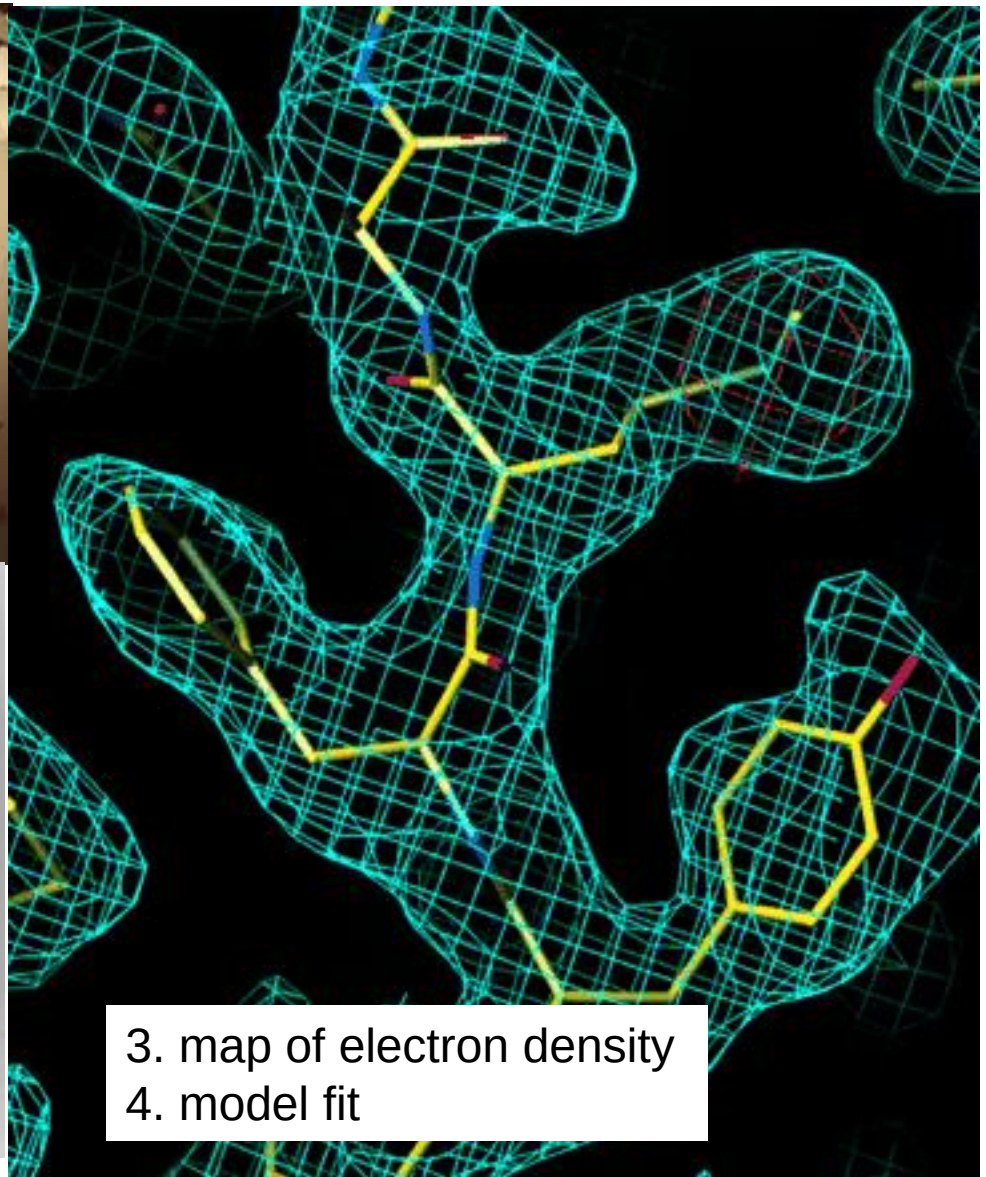
X-ray crystallography



1. protein crystals



2. diffraction experiment



3. map of electron density
4. model fit

Why X-Ray?

Elmag radiation interacts with objects of similar size with their wavelength (λ)

- visible light: λ = 350-700 nm and this is limit of optical microscopy

- RTG: CuKα: λ = 1,54 Å.

Synchrotron: λ = 0,5 Å – 2,5 Å.

atom-atom distances:

C-C = 1,54 Å,

C=C = 1,23 Å

C-N = 1,45 Å

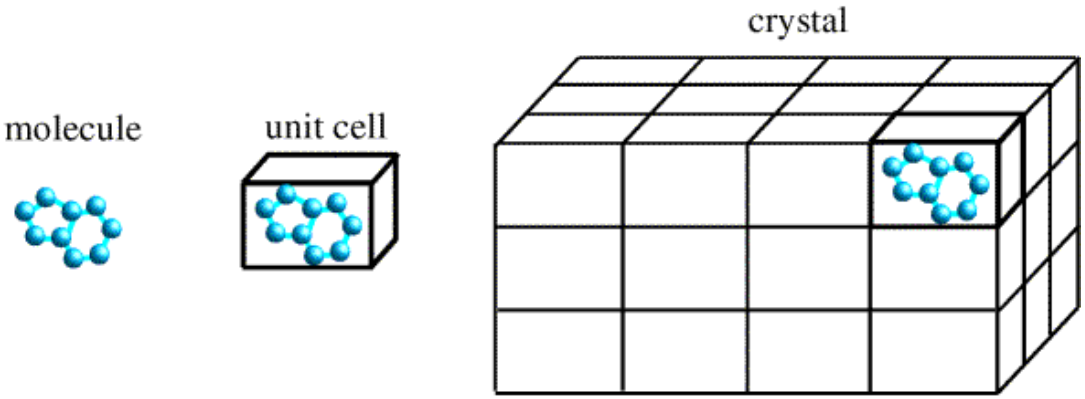
1 Å (Ångström) = 0.1 nm

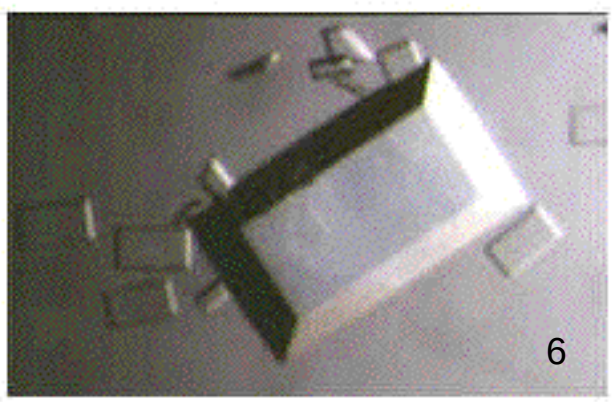
1 Å = 10^{-10} m

N-(H).....O = 2,8 Å

Why Crystals?

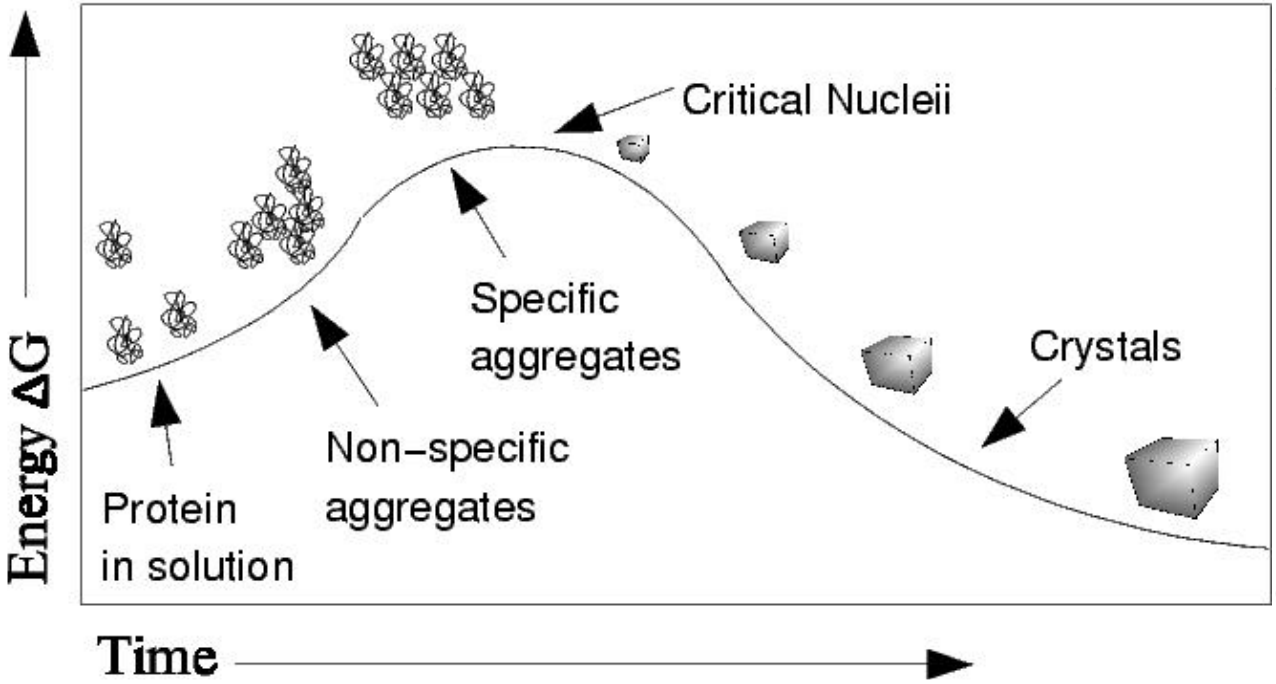
- X-ray diffraction on electrons (Bragg's law)
- One molecule=> small signal
- To increase signal-to-noise ration -> multiplication of signal => crystals of molecules in identical orientation



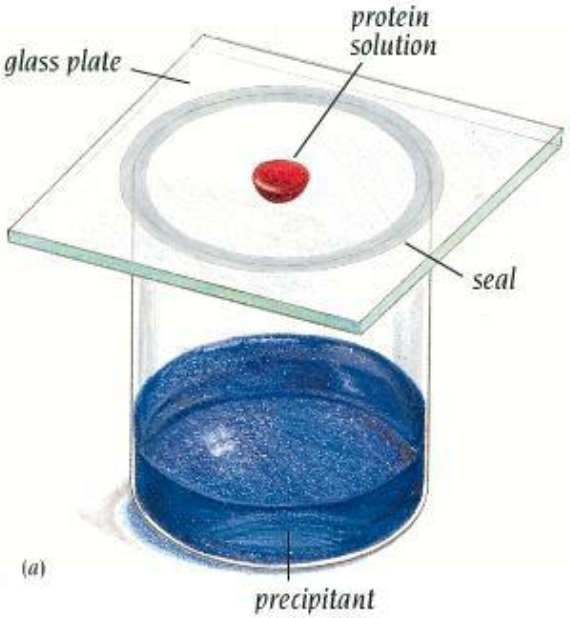


Crystallisation

Energetical diagram of crystallisation



Experiment – hanging drop



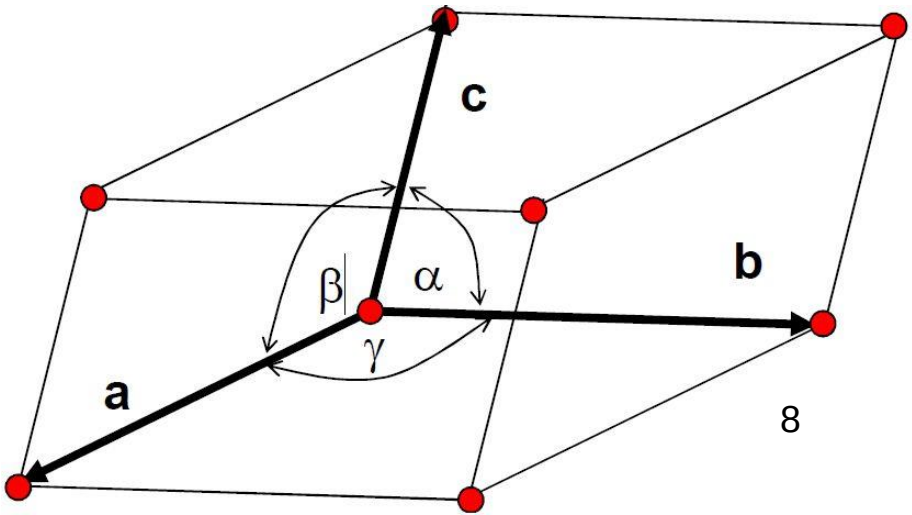
Airlie J McCoy, Protein Crystallography course
http://www-structmed.cimr.cam.ac.uk/Course/Crystals/intro.html

Crystal lattice

- Crystal have translation symetry from definition.
  - if ρ(**r**) – electron density in crystal point **r** => translational vectors **a, b, c** :

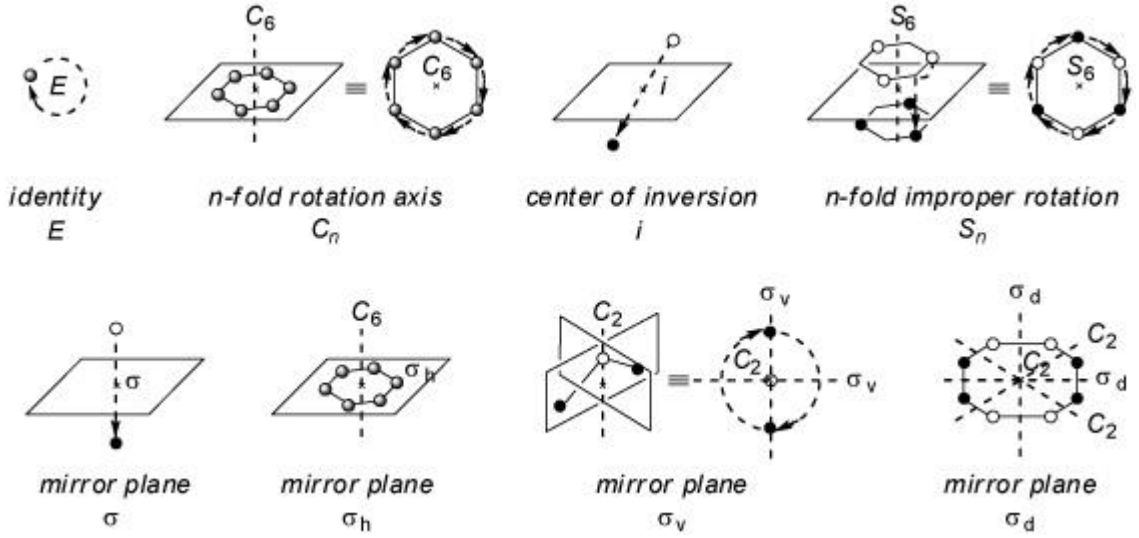
$$\rho(\mathbf{r}) = \rho(\mathbf{r} + u \cdot \mathbf{a} + v \cdot \mathbf{b} + w \cdot \mathbf{c})$$

  u, v, w are integers.
- Each identical copy – ***basic cell***.
- **a**, **b, c –** *unit cell vectors*.
- length of unit cell vectors is a = **|a|**, b = **|b|**, c = **|c|**.
- α, β, γ – angles between unit cell vectors
- Right-hand coordination system



Symmetry Operations

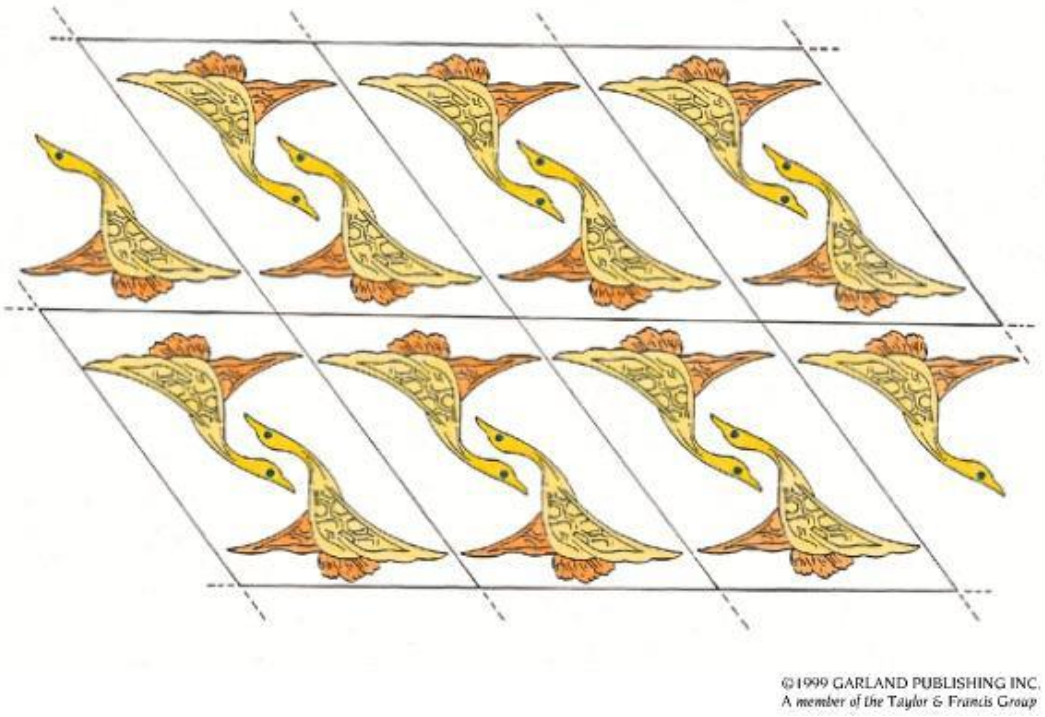
- translation
  (unit cell repetition)
- E – identity (C_1)
- i - inversion
  (point symmetry (S_2)
- C_n^m – rotational symmetry
  (rotation over axis 360°/*n* *m*-times)
- σ – mirror planes (S_1)
- S_n^m – combination of rotation 360°/n m-times followed with reflection over mirror plane
- All of above combined with translation



Assymetric unit and unit cell

assymetric unit

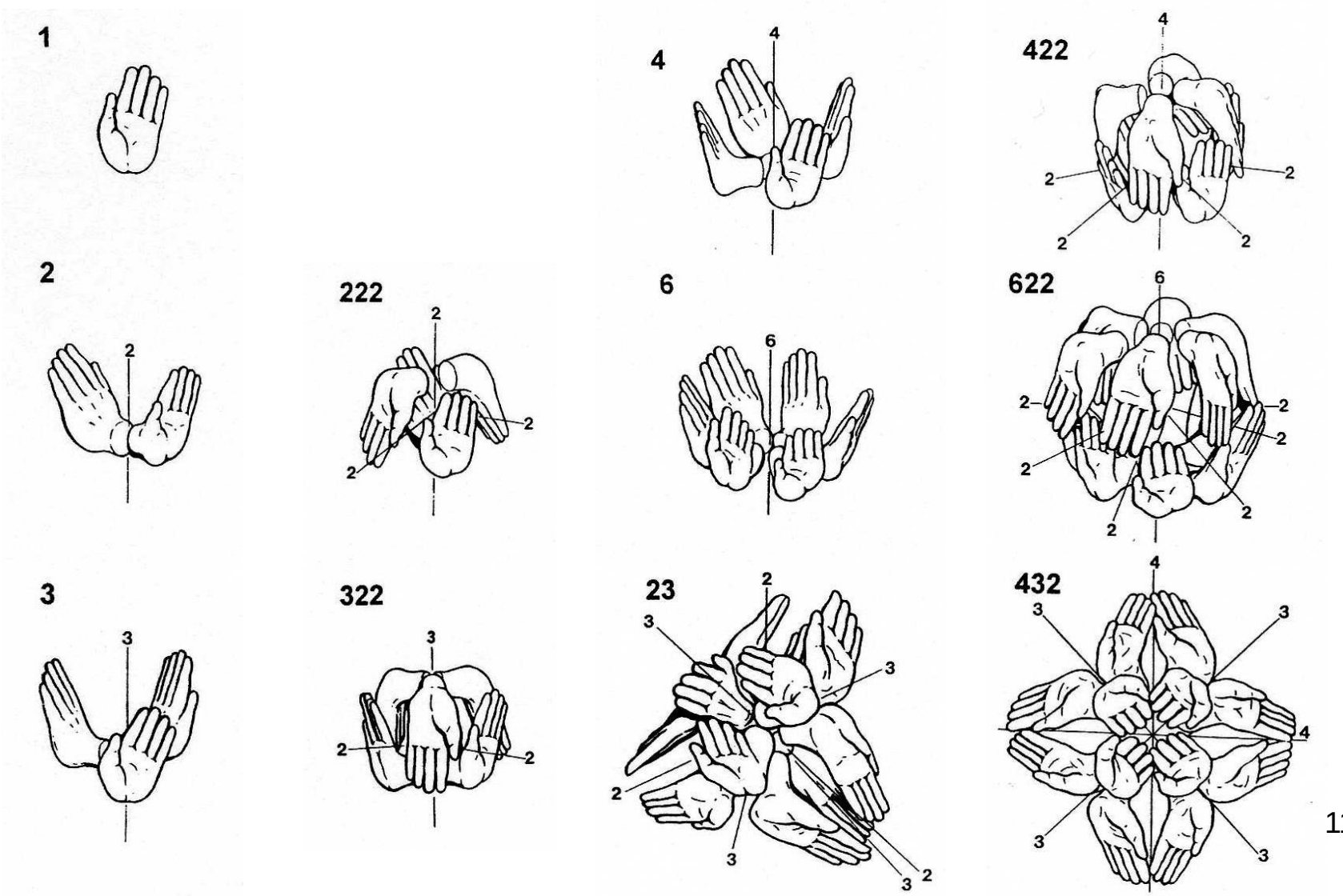
unit cell



Assymetric unit = smallest unit from which we can obtain unit cell with operations of symmetry

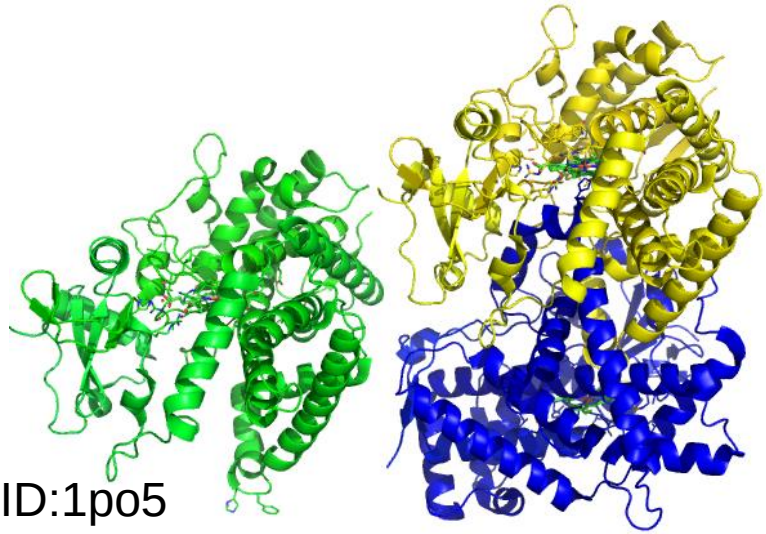
Unit cell = smallest unit which can be translated to whole crystal

Typical Point Groups of Symmetry of Protein Crystals



Crystal Contacts

- Protein crystals contain a lot of solvent
- Molecular contacts within crystal do not have usually (well not always) effect on protein structure



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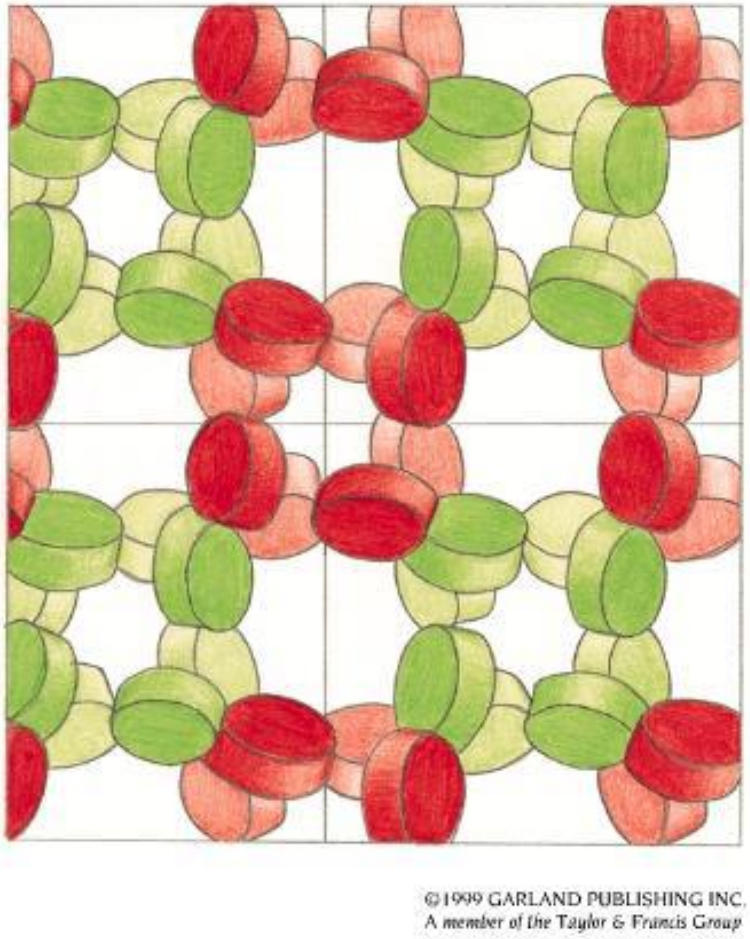
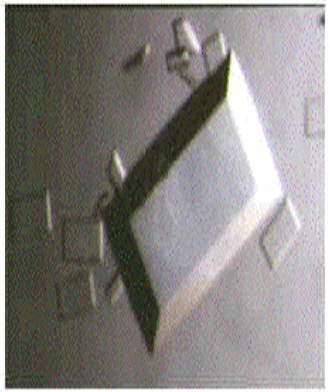
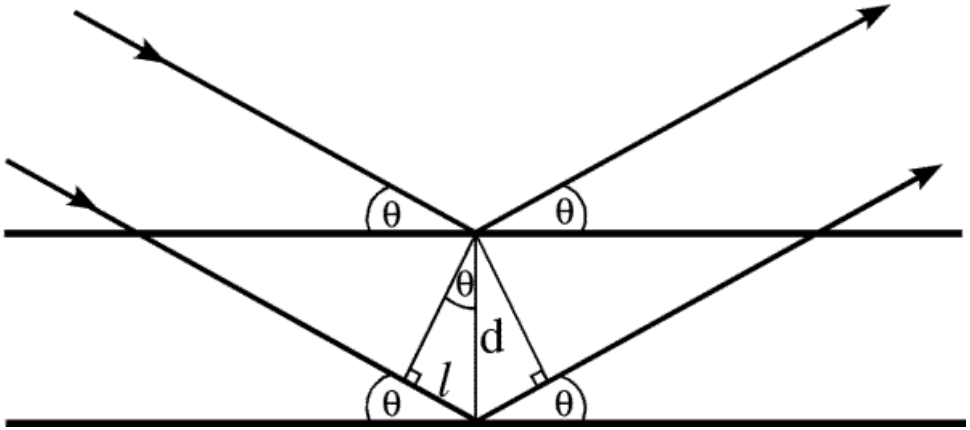


Packing of glycolate oxidase (schematically)

Diffraction Principle

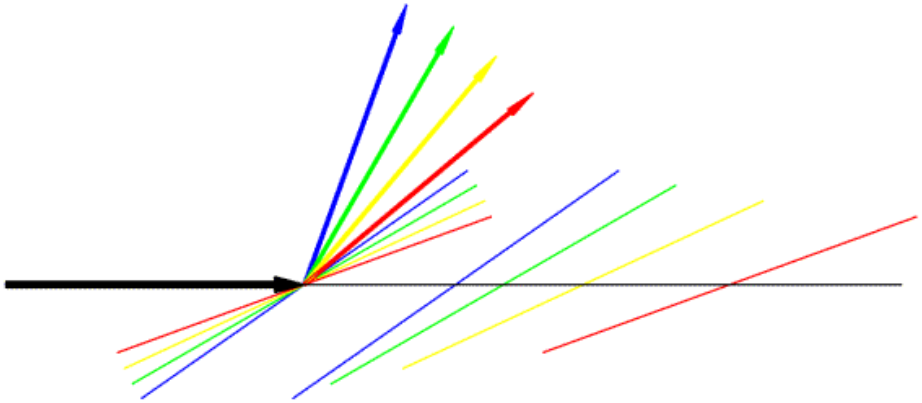




Bragg's law

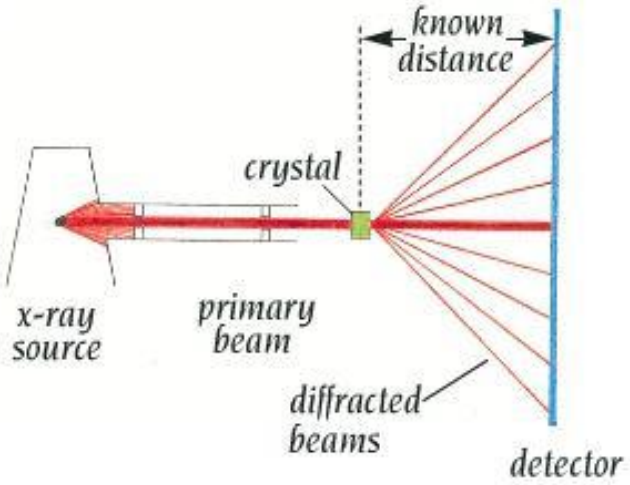
$n.\lambda = 2d.\sin\theta$

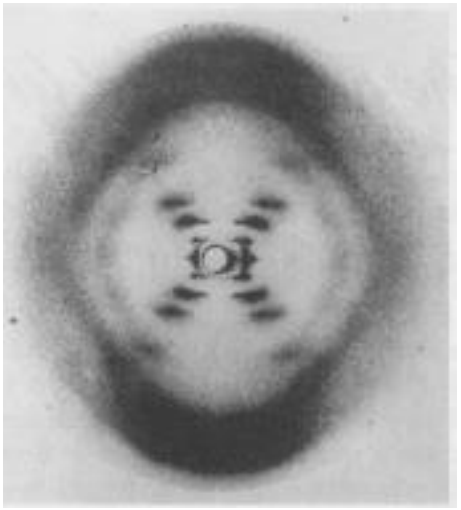
(W. H. Bragg & W. L. Bragg, 1912)



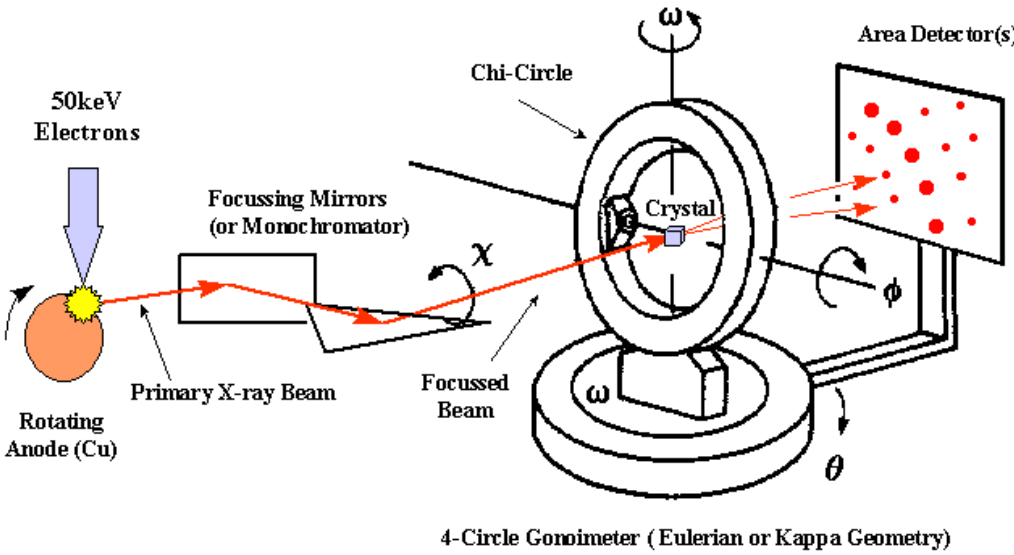
Diffracted radiation - sets of planes; parallel planes get boost in signal

X-ray Diffraction

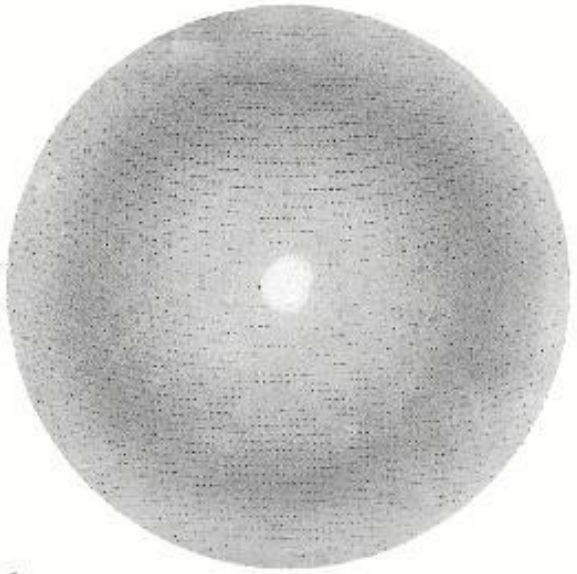




Rosalind Franklin & Raymond Gosling
Nature 171 (1953)

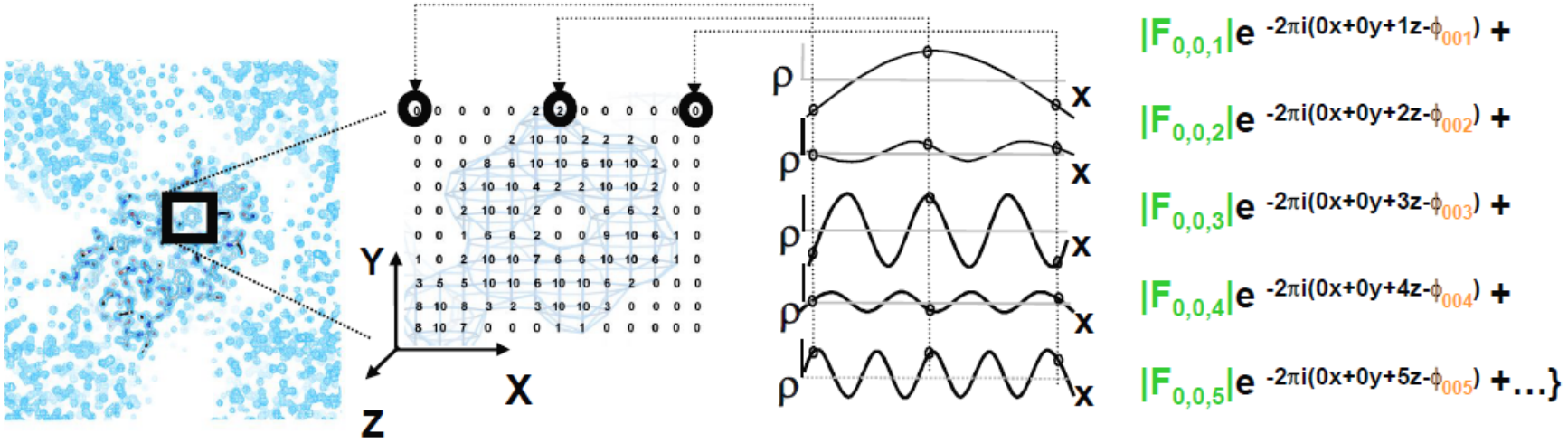


4-Circle Goniometer (Eulerian or Kappa Geometry)



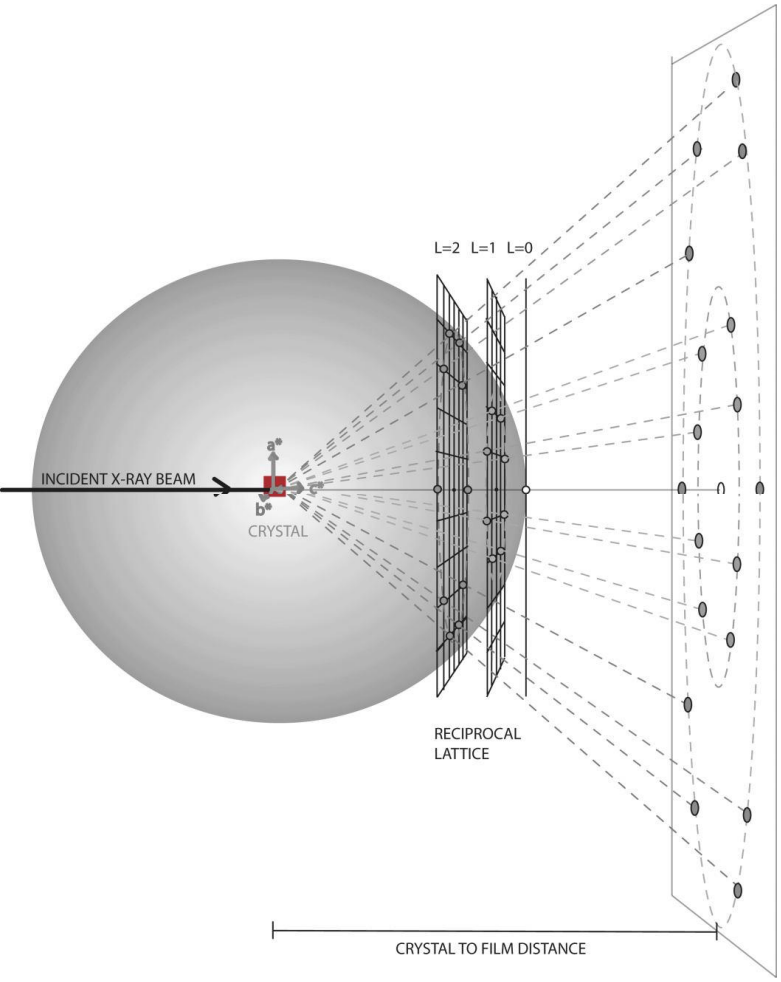
Calculation of Electron Density Map

Goal: use amplitudes and phases of thousands of diffractions F_{hkl} to calculate electron density map ρ(x,y,z)



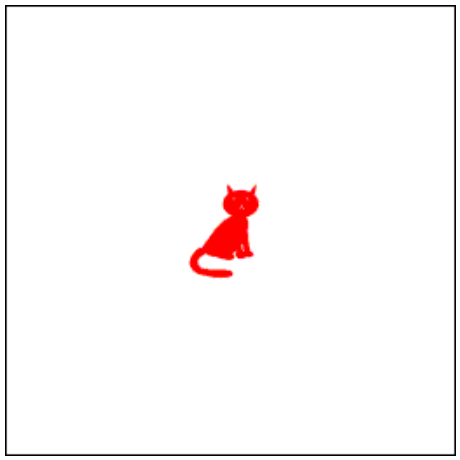
$$\rho(x,y,z)=1/v\sum_{h}\sum_{k}\sum_{l}|F_{hkl}|e^{-2\pi i(hx+ky+lz-\phi_{hkl})}$$

Phase Problem

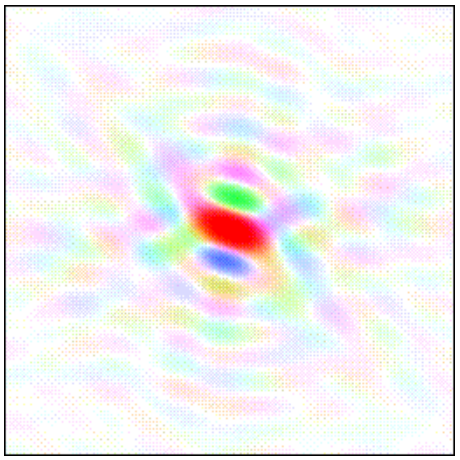


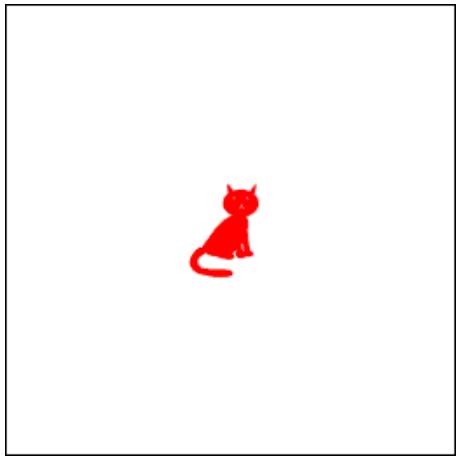
- Amplitudes and phases F_{hkl} are encoded in diffracted ray beams
- Amplitude $|F_{h,k,l}|$ is square root of intensity of diffracted beam.
- Φ_{hkl} is phase of diffracted wave. It cannot be directly measured – Phase problem.

Phase Problem Explained with Cats

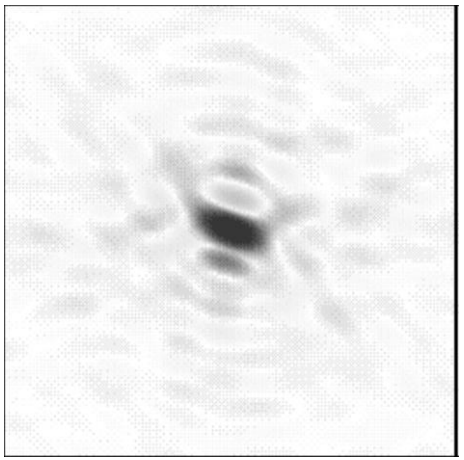


Diffraction data with phase information

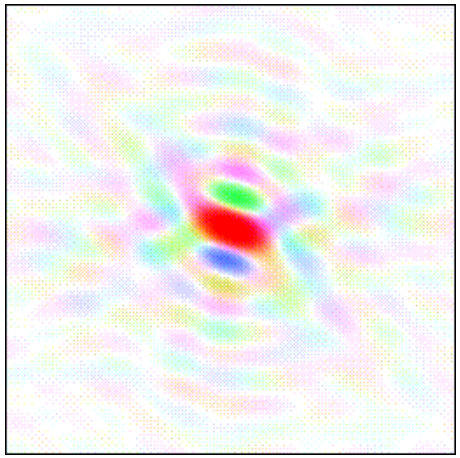




Real diffraction data

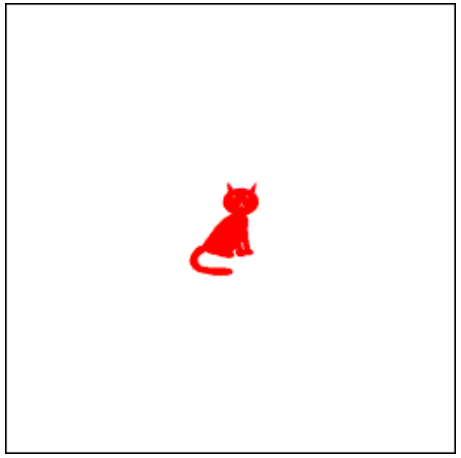


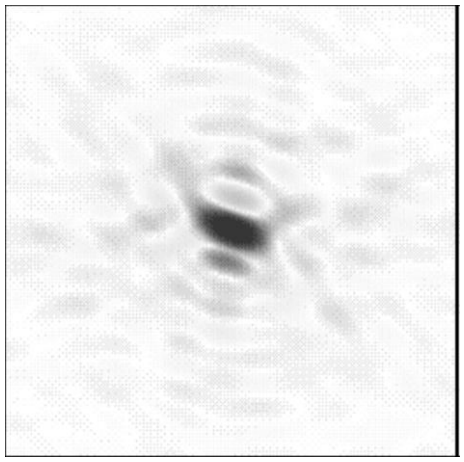
Reconstruction of Object



FT

easy





FT

hard

Phase Problem

John C. Kendrew

F_1, Φ_1

Max Perutz

F_2, Φ_2

F_1, Φ_2

F_2, Φ_1

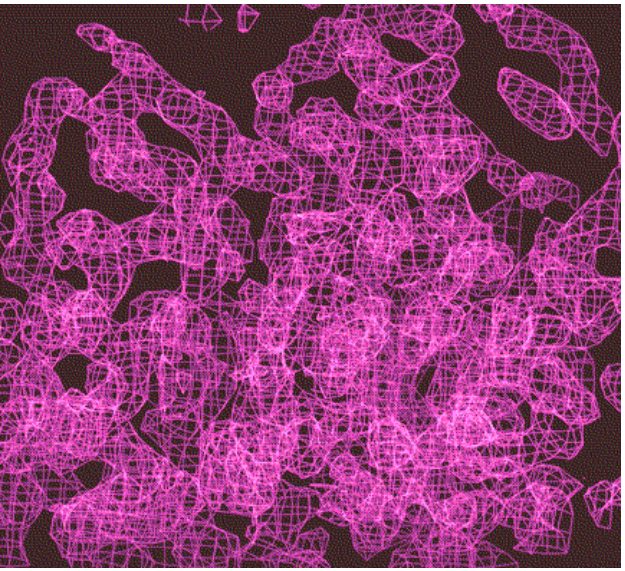
How to Solve Phase Problem

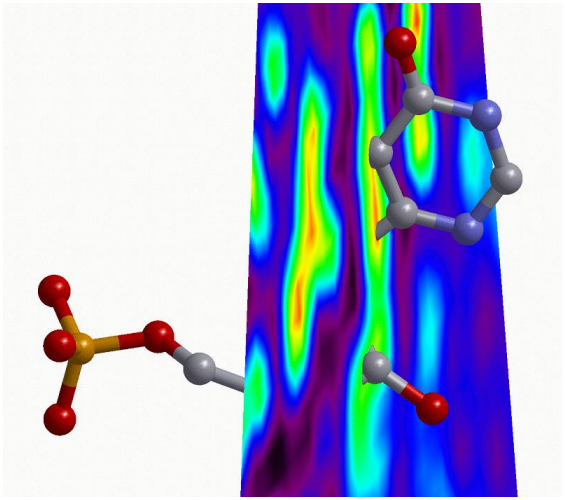
- **Direct methods**
  - Založeny na systematických souvislostech mezi určitými reflexemi.
  - Nutné mít data s vysokým rozlišením a relativně malý systém.
  - Velmi populární při řešení struktur malých molekul.
- **Molecular replacement**
  - Když existuje vyřešená struktura podobná té kterou chceme řešit,potom ji můžeme použít k odhadu fází.
  - Je stále více populární s tím jak roste počet vyřešených struktur.
- **Methods using Heavy atoms**
  - Do krystalu vneseme těžký atom, který silně rozptyluje (např. Hg, Fe, Pb, I,Se ..). Nebo nahradíme všechny Met v proteinu Se-Met.
  - Použijeme vícečetné isomorfní nahrazení (Multiple Isomorphous Replacement).MIR
  - Použijeme vícečetnou nebo jednoduchou anomální difrakci (Multiple nebo Single Anomolous Diffraction). MAD

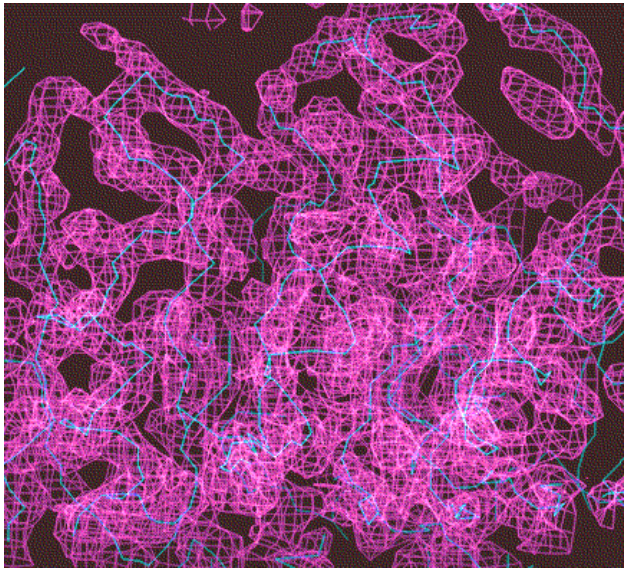
there is an app for that… SOLVE, SHELL-X, Phaser…

First Electron Density Map

- Phase and Amplitudes => Fourrier transformation => electron density map
- Fitting of model
  - Cα atoms using poly-Ala.
  - identification of secondary structural elements
  - use of sequential information



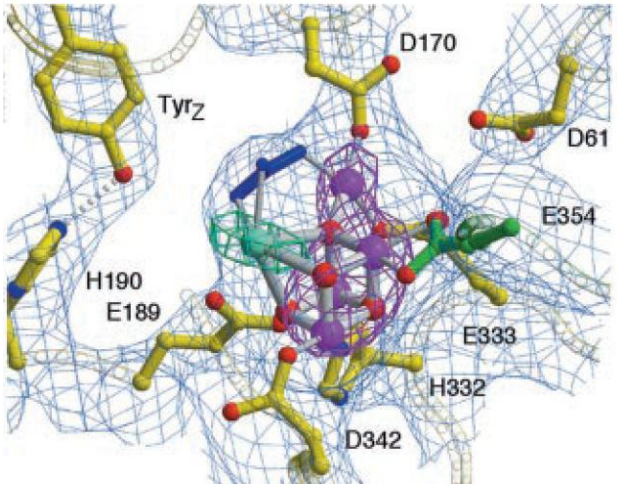




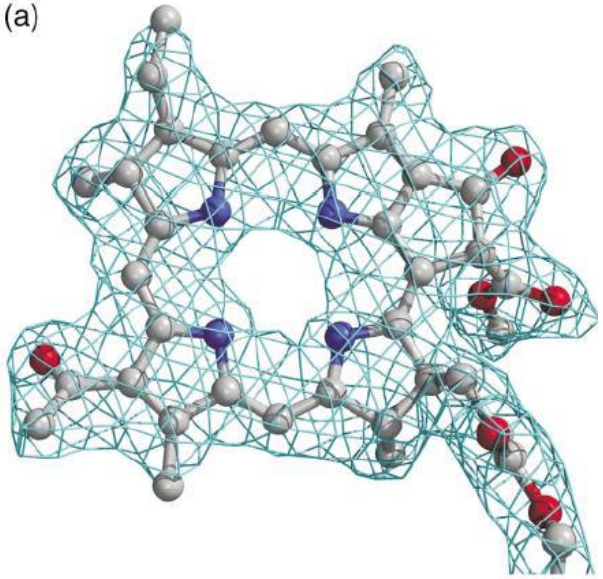
- Programs: CNS, O, COOT, ARPwARP

Resolution (R)

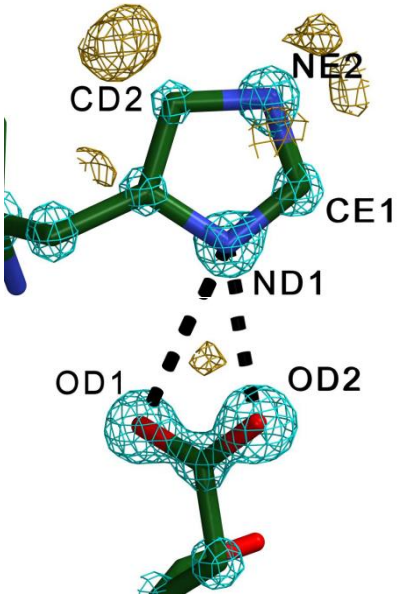
- in Å,
- Distance for distinguishing of two points. Details should be distinguishable at 0.7·R.
- better R – easier model building!
- (more refflections – better signal-to-noise ratio)



3.5 Å map of photosystem II



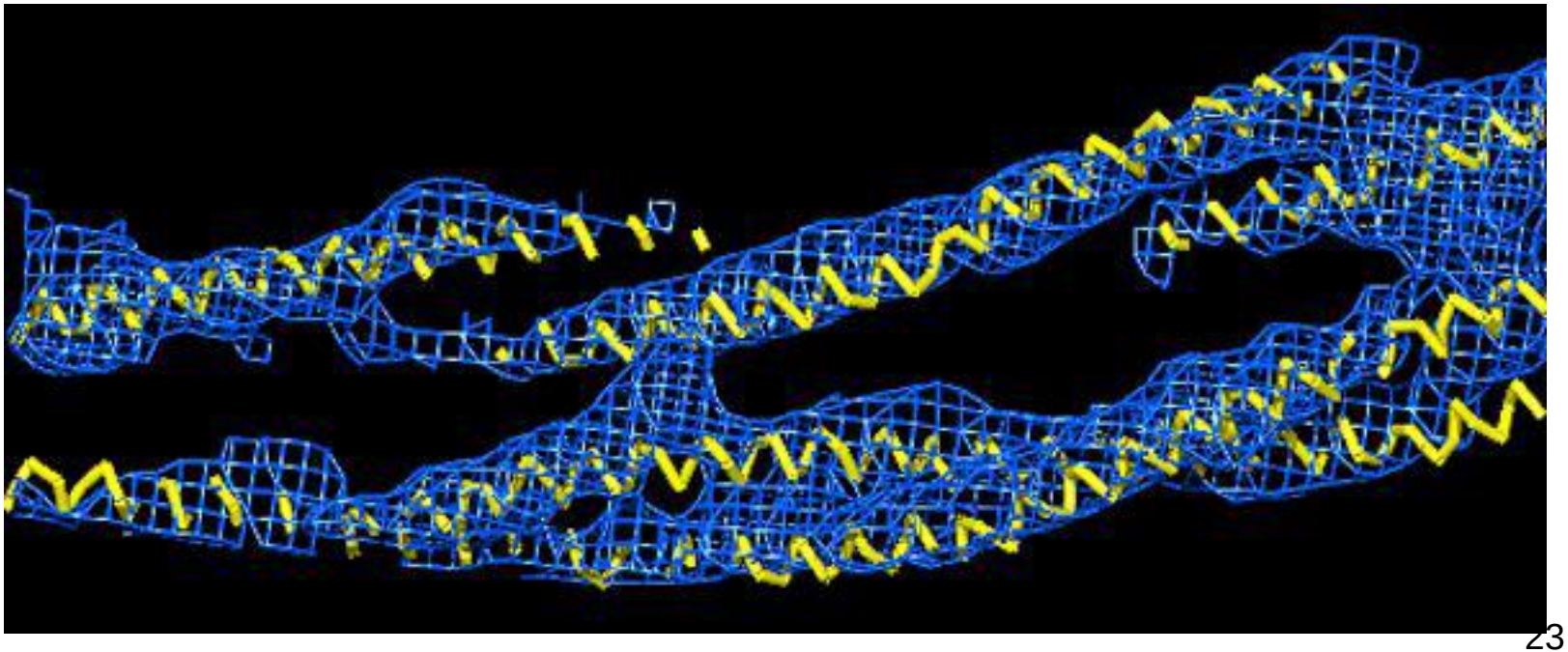
2.3 Å map of photosynthetic reaction centre



0.95 Å map of elastase

Low Resolution

- Mapy s nízkým rozlišením ukazují pouze obecné vlastnosti jako je např. tvar molekuly a umístění elementů sekundární struktury.

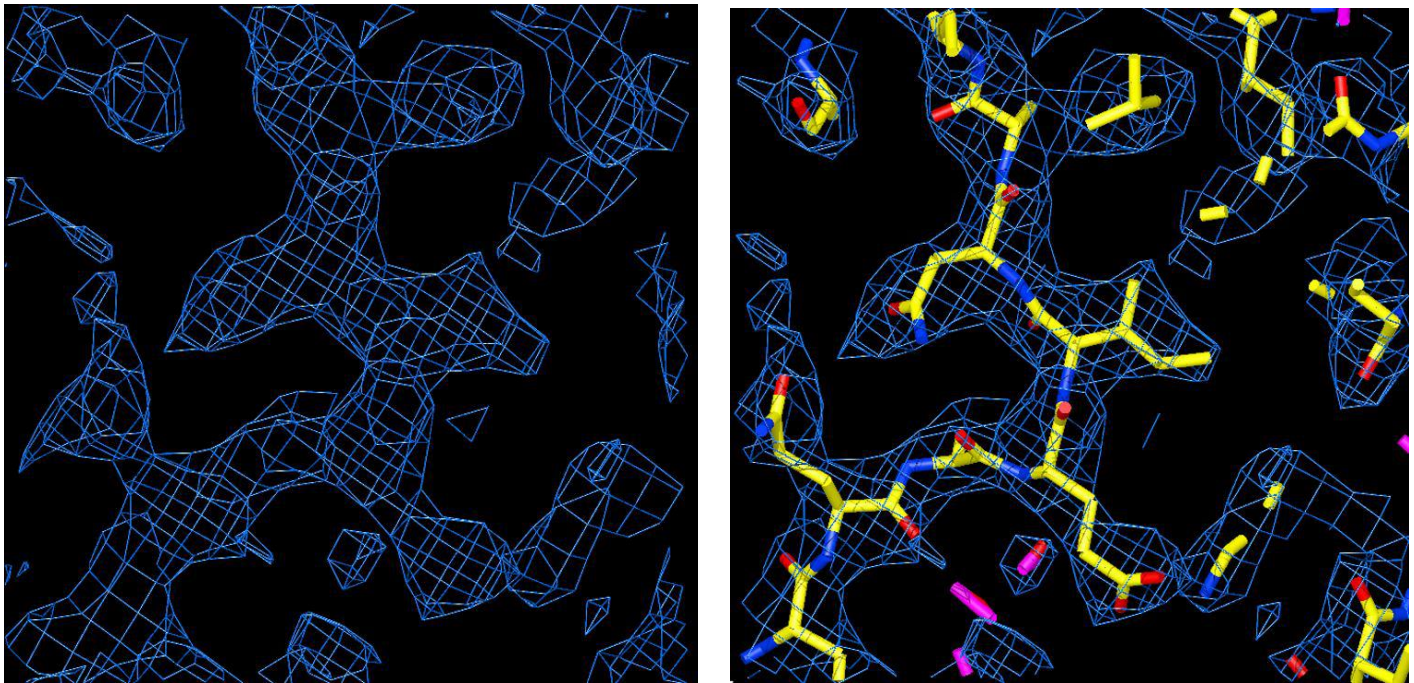


R = 7 Å, tropomyosin.

Klasické rozlišení proteinů

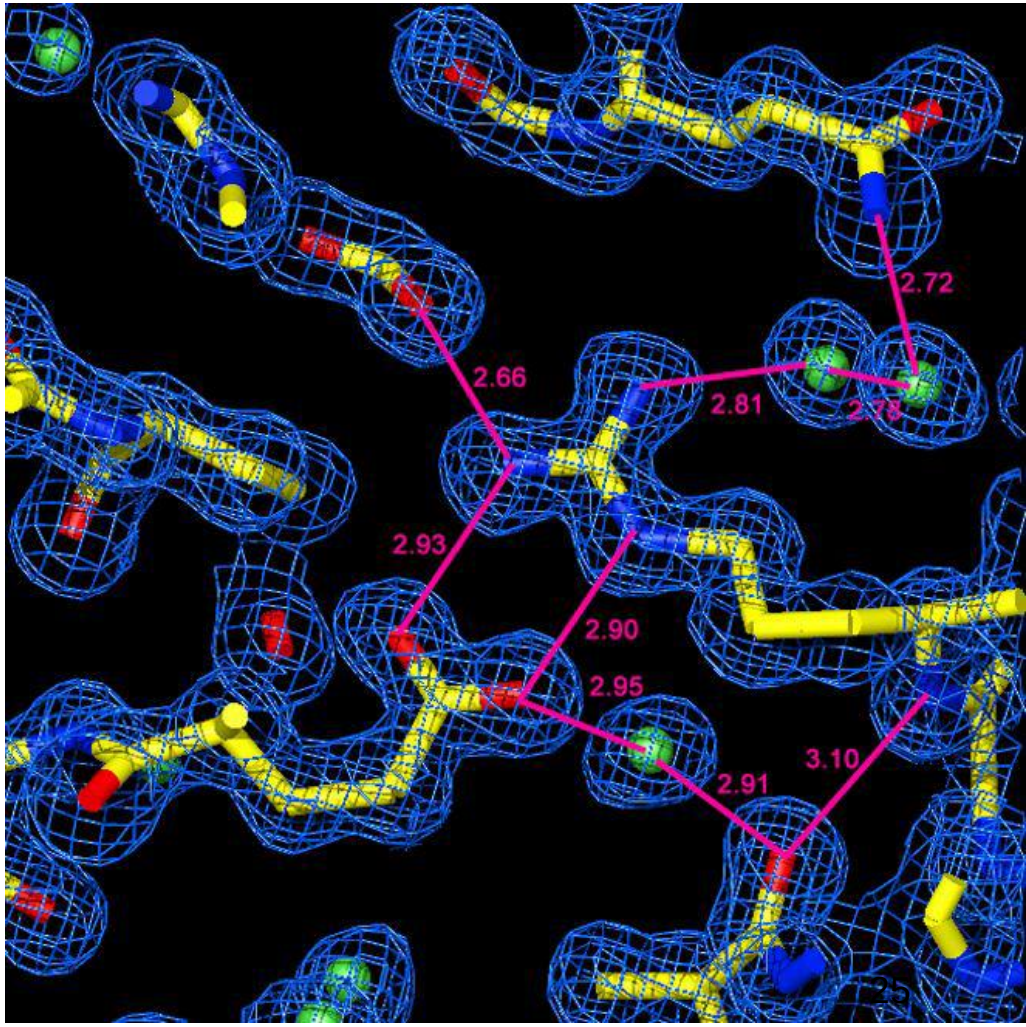
- běžné rozlišení u proteinových struktur pod 2.5 Å
- Při tomto rozlišení je snadné sledovat průběh hlavního řetězce a řada postraních řetězců má také dobře definovanou hustotu. U proteinů je limit pro publikaci struktury rozlišení 3.0 Å.

R = 2.6 Å



Vysoké rozlišení

- Mapa elektronové hustoty s velmi vysokým rozlišením jasně ukazuje pozice jednotlivých atomů.



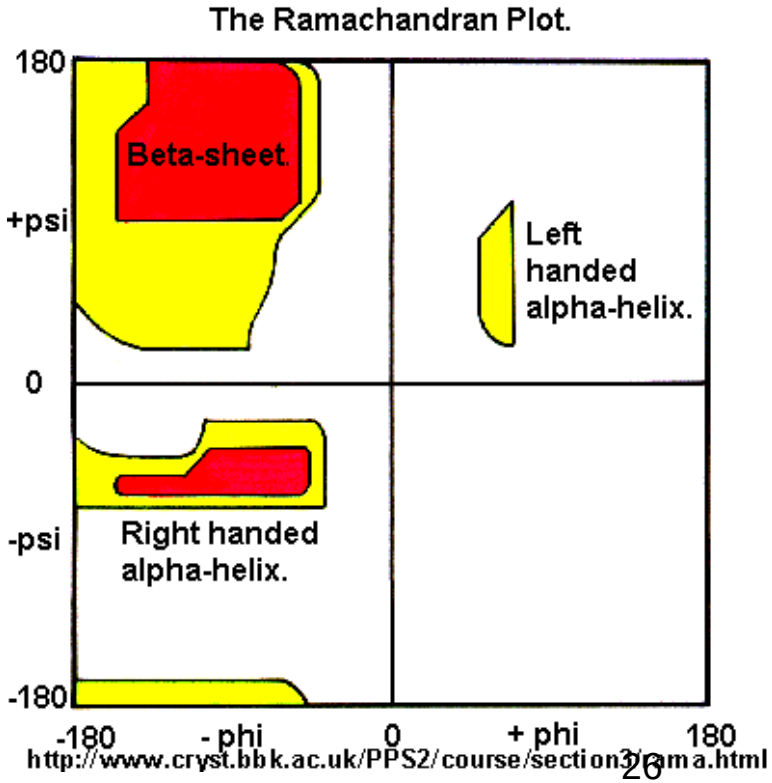
R = 1.2 Å
H-vazby (fialová) mezi N a O atomy.

Validace

- R_{free} (Brünger, 1992)
  - test set (~5-10%) of reflekcí vynecháno z určování struktury

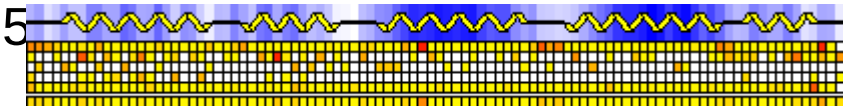
- Stereochemie

Ramachandranův diagram
  - WHATIF
  - MOLPROBITY
- Bad contacts
  - stérické problémy ve struktuře



kontrola kvality

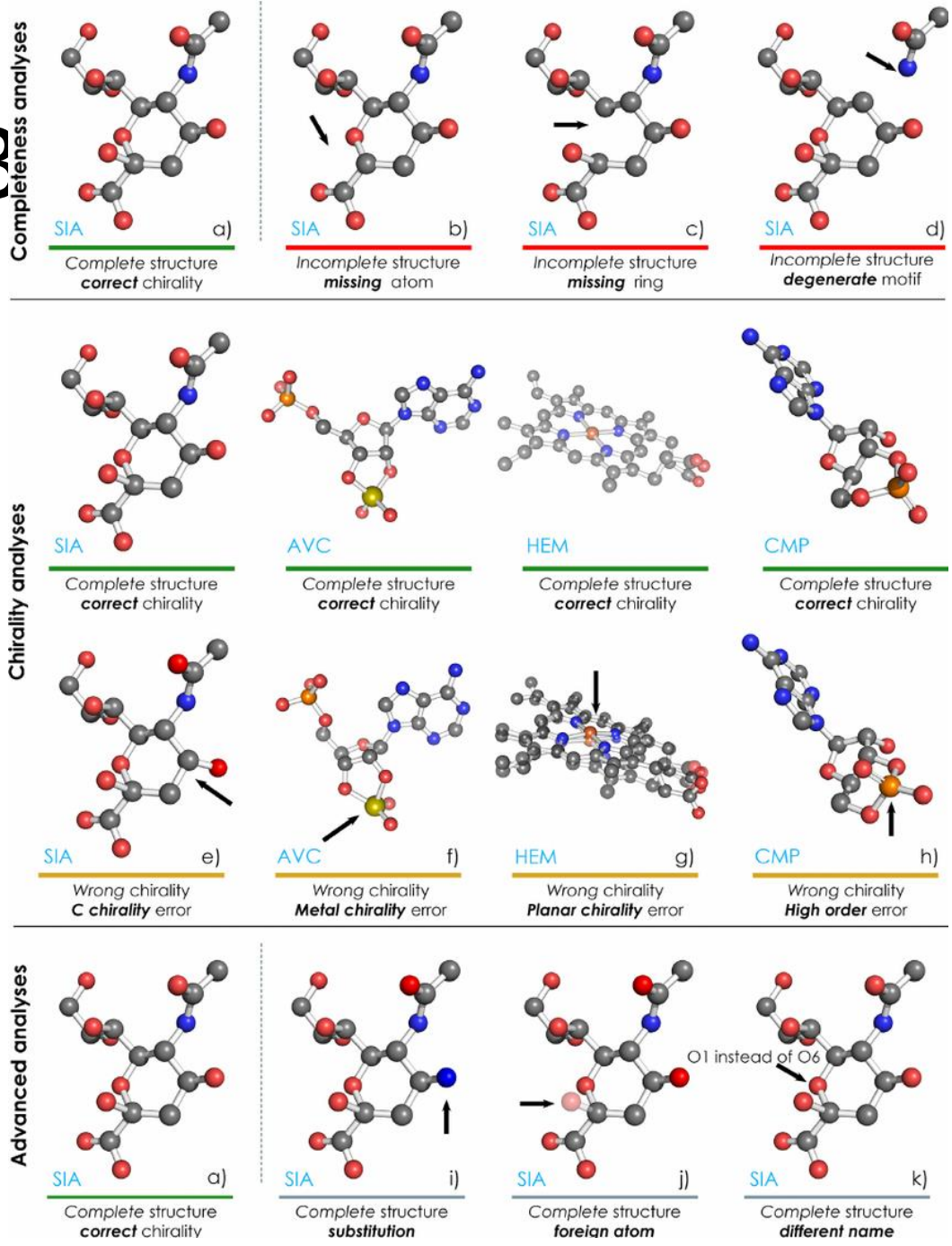
- Model quality is assessed with Knowledge-Based (KB) and Model-vs-Data (MvD) metrics
  - KB metrics compare structural features **with the database of protein structures**
  - MvD metrics **compare models against experimental data**
- Assessment requires annotation of well-defined regions the protein structure model
- Stereochemická – PROCHECK 3.5



  - 1. Ramachandran plot
  - 2. Ramachandran plots by residue type
  - 3. Chi1-Chi2 plots
  - 4. Main-chain parameters
  - 5. Side-chain parameters
  - 6. Residue properties
  - 7. Main-chain bond length distributions
  - 8. Main-chain bond angle distributions
  - 9. RMS distances from planarity
  - 10. Distorted geometry plots
- R_{free} – cross-validace - 5-10 % dat vynecháno.

ValidatorDB

- Ligandy
- kontrola oproti DB
  - kompletnost
  - kontrola chirality
  - substituce



Vařeková,R.S., Jaiswal,D., Sehnal,D., Ionescu,C.-M., Geidl,S., Pravda,L., Horský,V., Wimmerová,M. and Koča,J. (2014) MotiveValidator: interactive web-based validation of ligand and residue structure in biomolecular complexes. Nucleic Acids Research, 42(Web Server issue), W227–33.

And now something completely different…

NMR

aneb

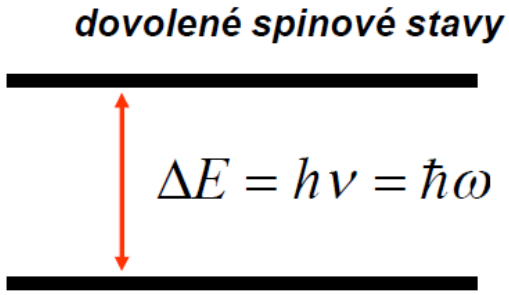
Nukleární magnetická rezonance

Nukleární magnetická rezonance NMR

- NMR spektroskopie využívá magnetických vlastností jader atomů.
- Absorpční(emisní) spektroskopie, podobně jako IČ nebo UV. Detekuje absorbci radiofrekvenčního záření jádry atomů v molekule.
- Radiofrekvenční energie (ΔE přechodů jaderného spinu):

λ = 1011 až 3 x 107 nm

v = 106 až 1010 Hz

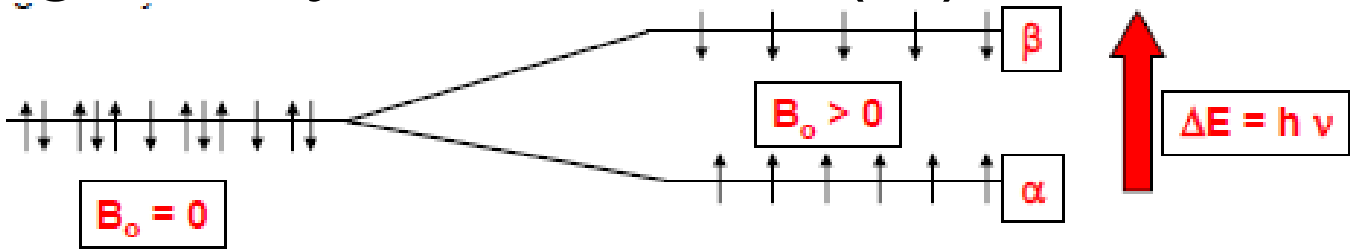


Nastavením frekvence elektromagnetického záření (v, nebo ω) na rezonanční podmínku dojde k indukci přechodů mezi hladinami jaderného spinu

(*tzn.* můžeme měřit NMR spektrum!).

NMR

- Externí magnetické pole => energetický rozdíl mezi spinovými stavy jaderných magnetických momentů (m)



- Rozdíl v populaci stavů je dán rozdílem energií

$$N_\alpha / N_\beta = e^{\Delta E / kT}$$

- např. $\Delta E = 3.8 \times 10^{-5}$ kcal/mol pro ^1H při 400 MHz (B_o=9.5T) Nα/Nβ= 1.000064
- Rozdíly populací velmi malé

Pravidla pro určení spinu izotopu

Nukleon.č.(A)	**Proton.č.(Z)**	**I**	**Detekce**
liché	sudé nebo liché	1/2, 3/2, 5/2	ano
sudé	sudé	0	ne
sudé	liché	1, 2, 3	ano

Možný počet spinových stavů = 2I + 1:

Jádro	Spin.kvant.č.	Počet stavů	Mag.spin.č.
^{1}H	I = 1/2	2(1/2) + 1 = 2	m = ±1/2
^{14}N	I = 1	2(1) + 1 = 3	m = -1, 0, 1

NMR-aktivní jádra v proteinech

- Přirozená:

  ^{1}H, spin ½

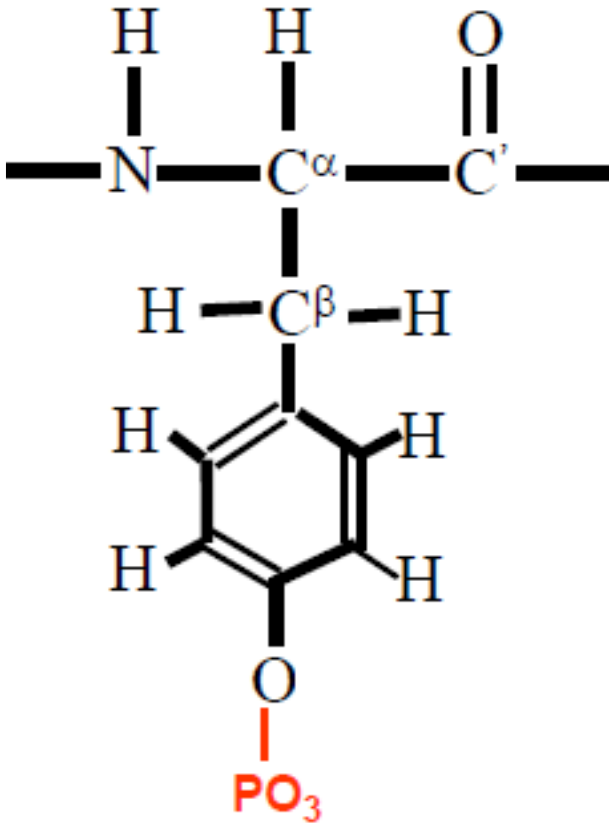
  ^{31}P, spin ½

- Obohacená díky bakteriální expresi:

  ^{2}H, spin 1

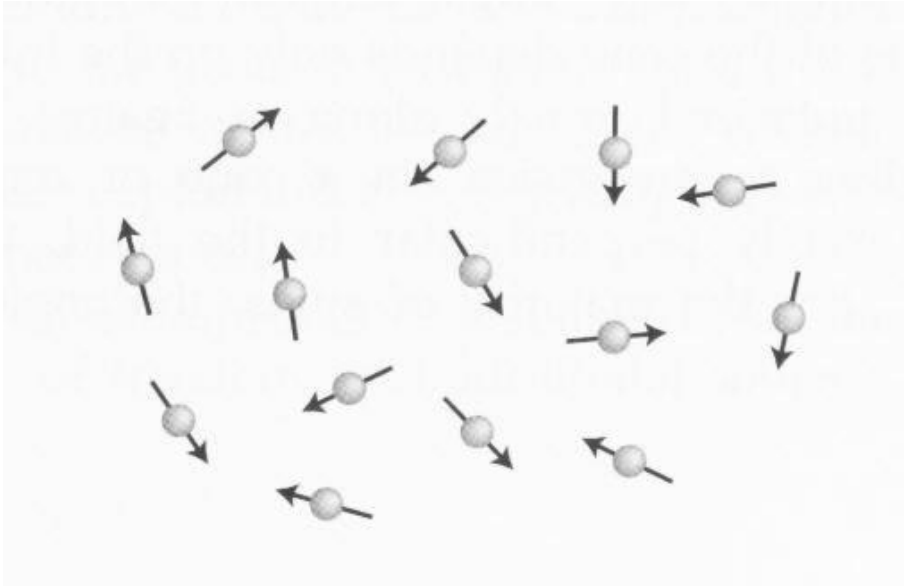
  ^{13}C, spin ½

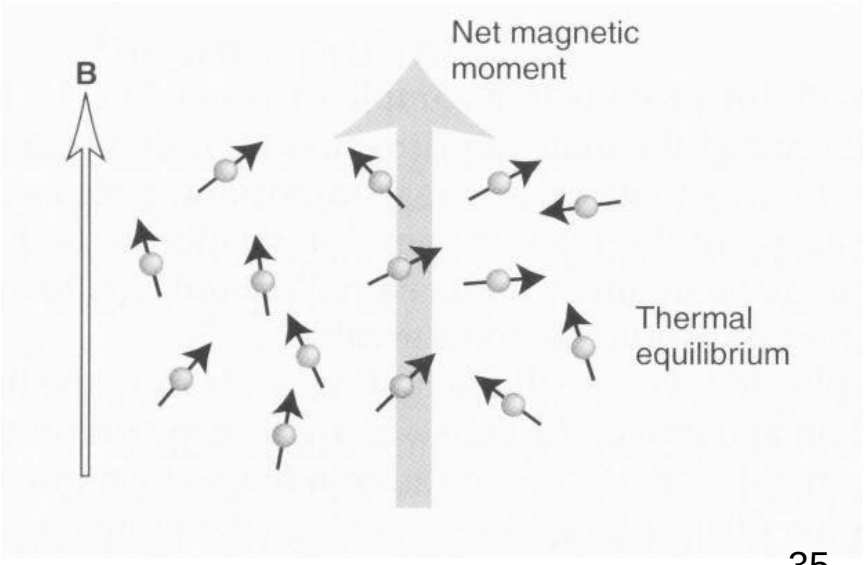
  ^{15}N, spin ½



Orientace mag.spinů

- Bez externího magnetického pole
  - náhodné orientace
  - degenerace
- S magnetickým polem
  - Makroskopická magnetizace **(M_z)**





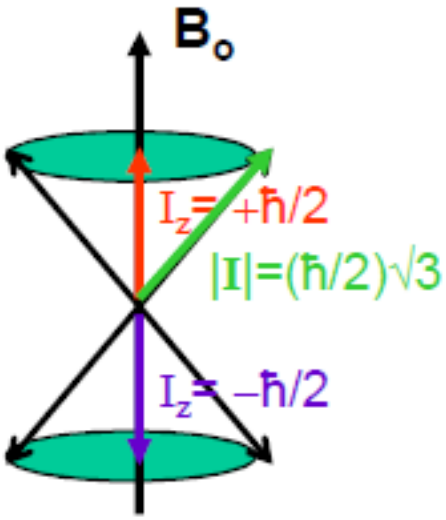
Vliv magnetického pole

Energie μ v poli B_o

$E = - \mu \cdot B_o$

pro jednu hladinu:

$E_m = -m \cdot B_o \cdot \gamma \cdot \hbar$

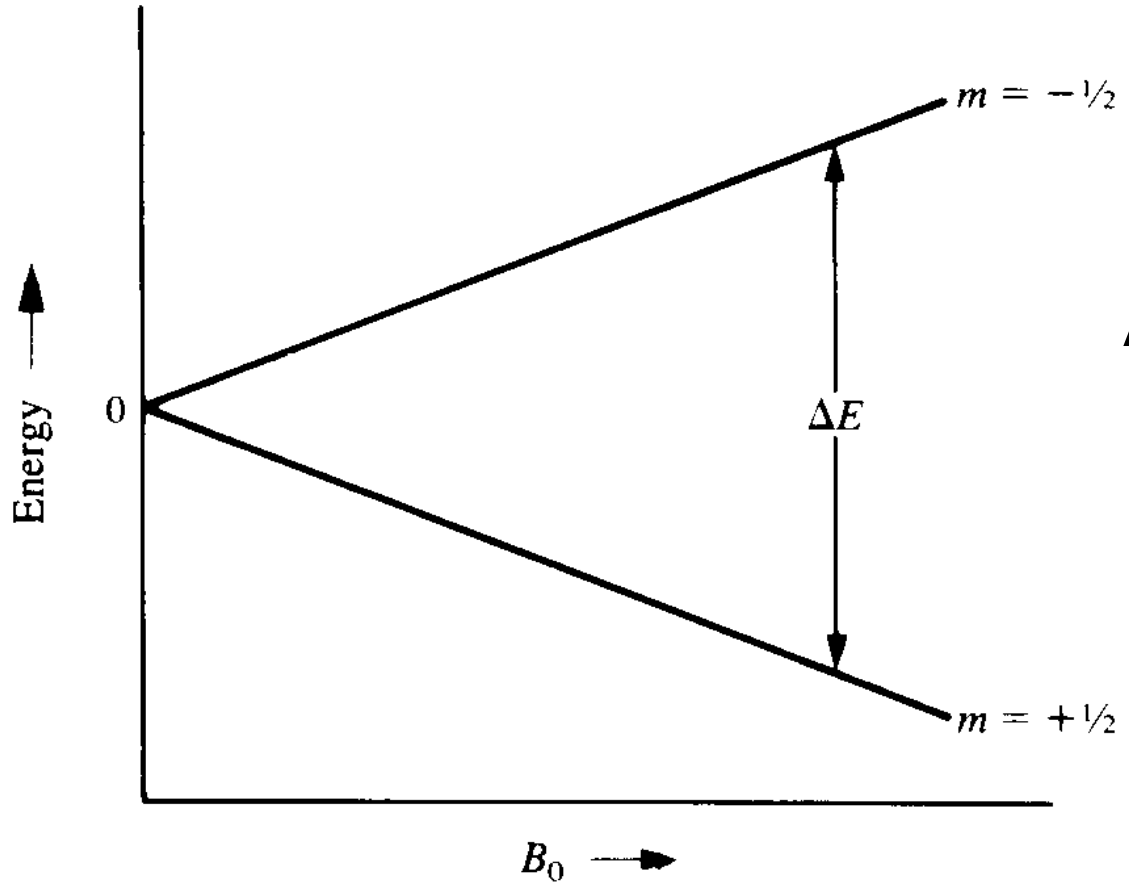


Projekce je kvantována, takže *E* závisí na:

1) typ jádra (*γ*)
2) spinový stav (*m*)
3) síla magnetu (B_o)

Rezonance

rezonanční podmínka: $\Delta E = h\nu = \hbar\omega = B_o\gamma\hbar$

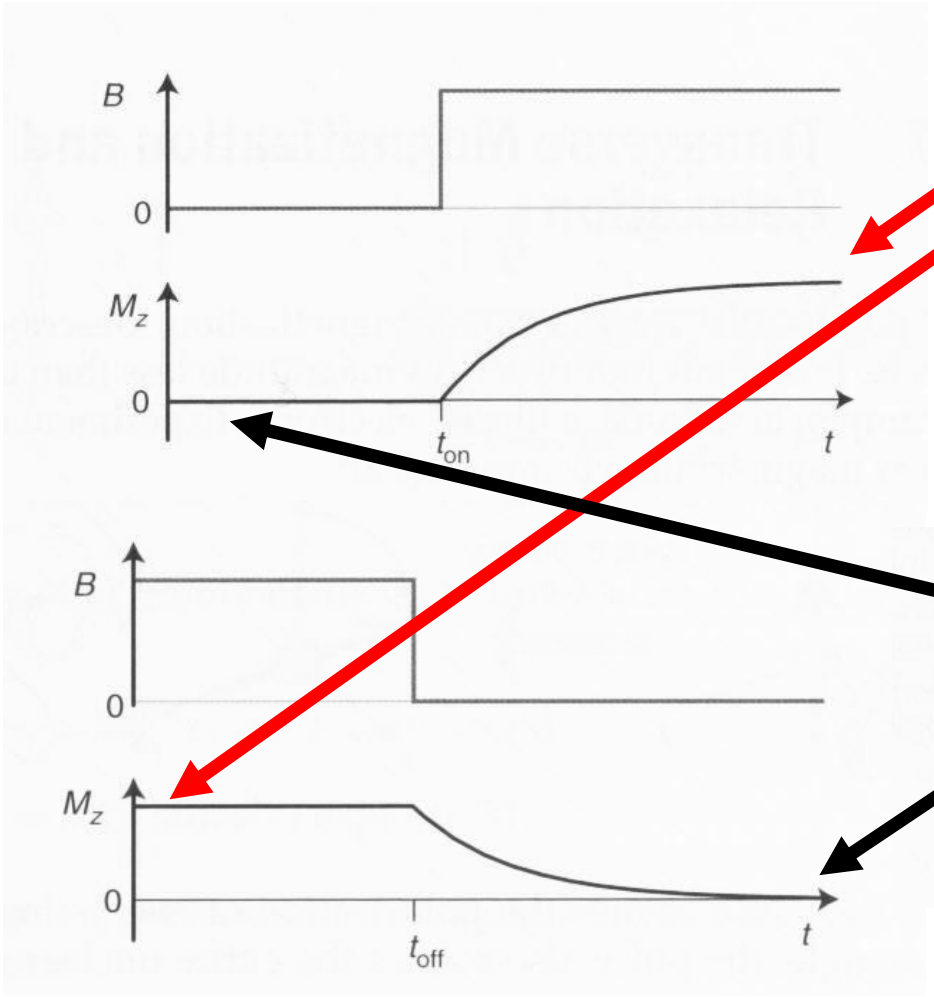


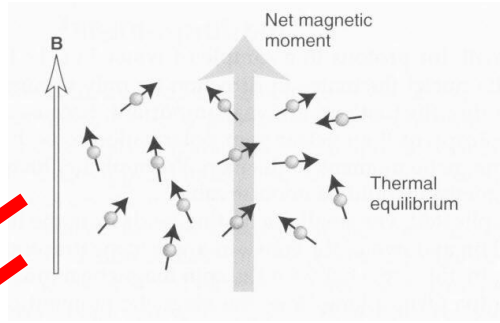
$\Delta E = \hbar\omega_o$

$\omega_o = B_o\gamma$

Excitace a podélná relaxace (M_z -> rovnováha)

- nárůst
- pokles

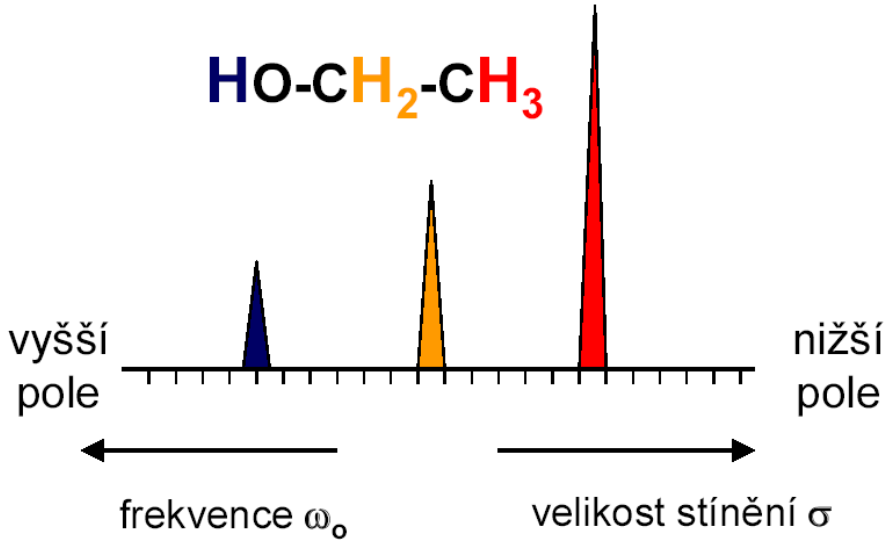




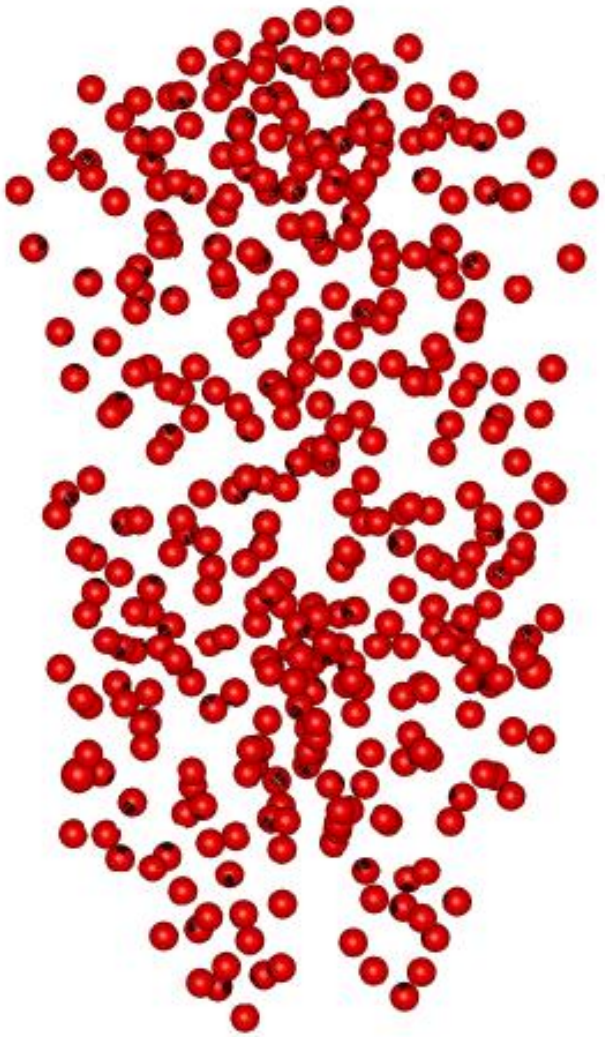
Chemický posun

- Různá jádra mají různé rezonanční frekvence
- Magnetické pole, ve kterém se jádro nachází není rovno vnějšímu magnetickému poli.
  - Elektrony v okolí jádra (chemické okolí) stíní vnější pole – výsledné efektivní magnetické pole $\mathbf{B_{eff}}$ je tvořeno vnějším polem $\mathbf{B_0}$ a polem lokálním $\mathbf{B_{loc}}$.

$\mathbf{B_{eff} = B_0 – B_{loc} = B_0(1-\sigma)}$, kde σ je konstanta magnetického stínění

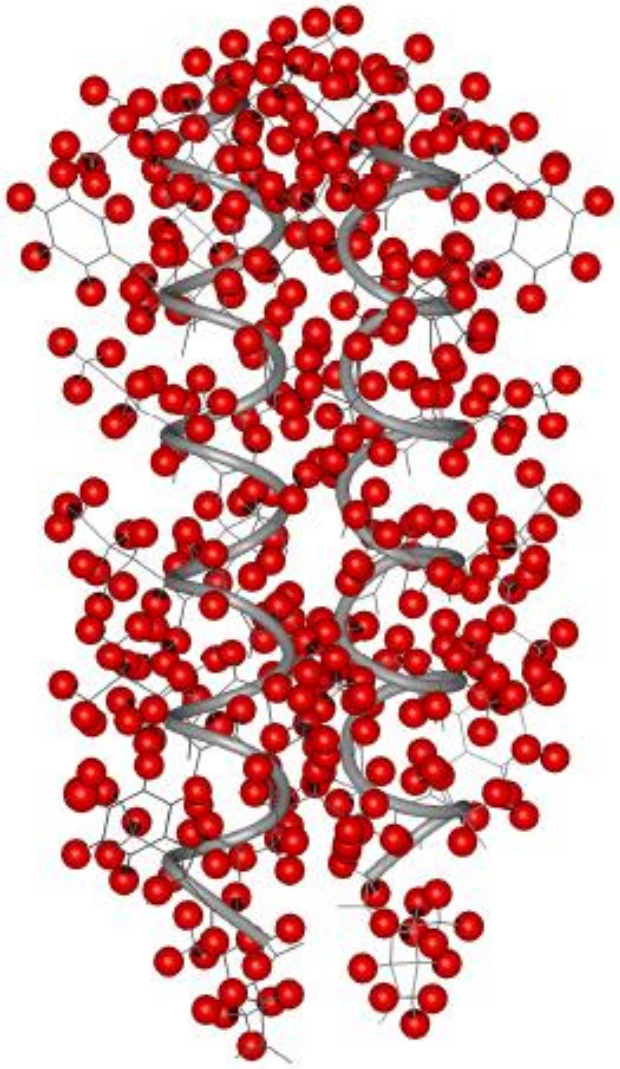


NMR – řešení struktury makromolekul



- Pozorujeme protony (^{1}H)
  - (! x-ray difrakce, kde elektronová hustota atomů s vyšším počtem elektronů (C, N, O)!)
- Protein je v roztoku.

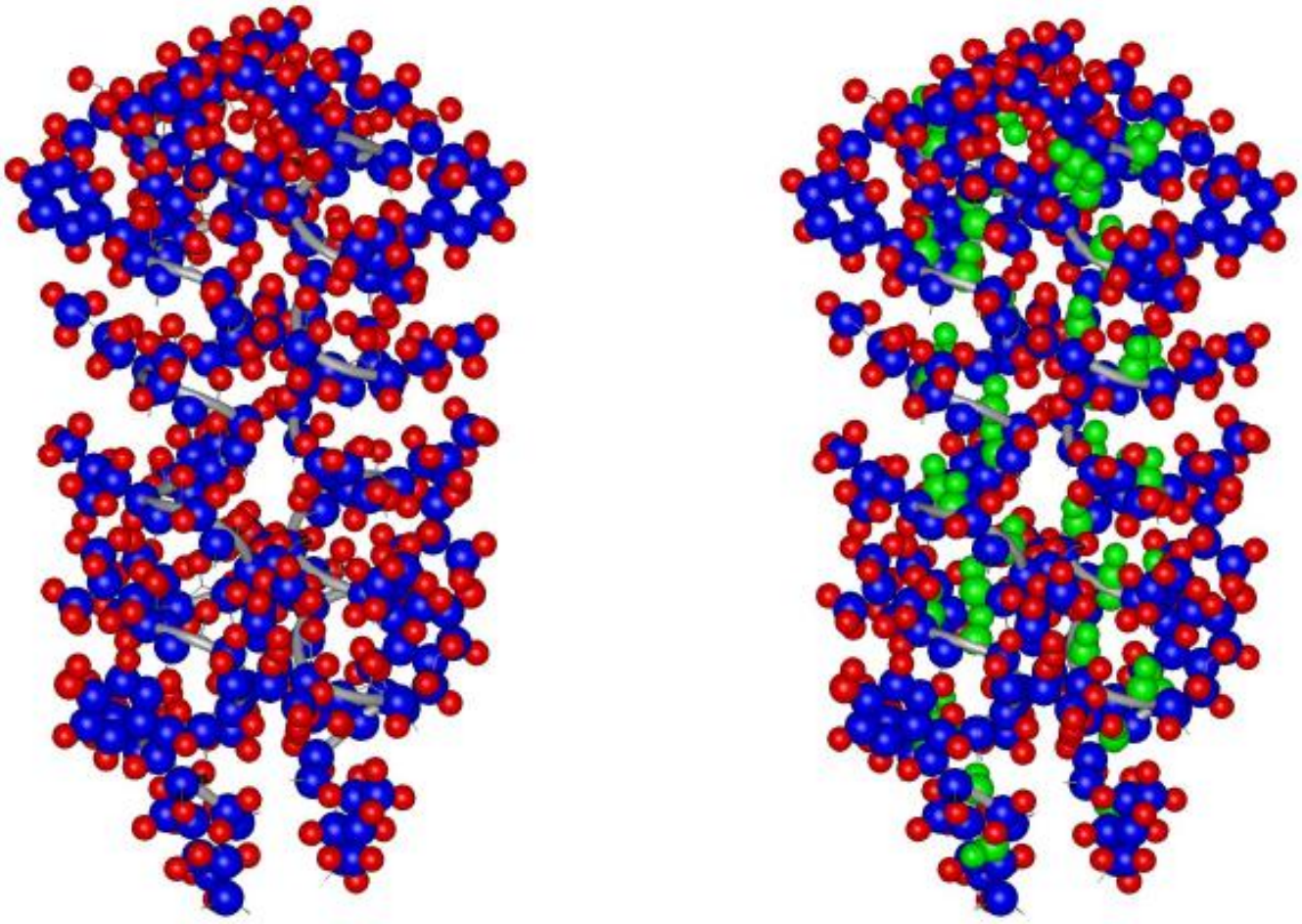
NMR proteinů



- Přiřazení pozorovaných ^{1}H rezonancí individuálním aminokyselinám.
- Protonové rezonance jsou často rozlišeny na základě rozdílů v chemickém posunu.
- Měříme intra-aminokyselinové a inter-AK proton-protonové vzdálenosti prostřednictvím dipolárních spřáhnutí.
- Měříme torzní úhly prostřednictvím J- spřáhnutí.
- Získané vzdálenosti a torzní úhly použijeme k určení sekundární a terciární struktury.

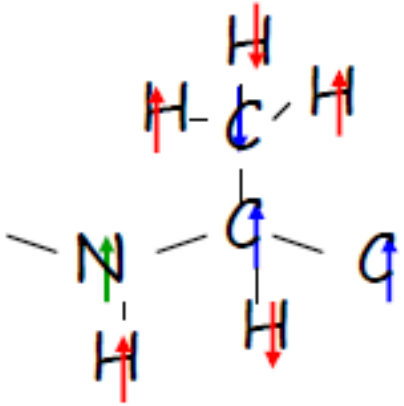
NMR interakce

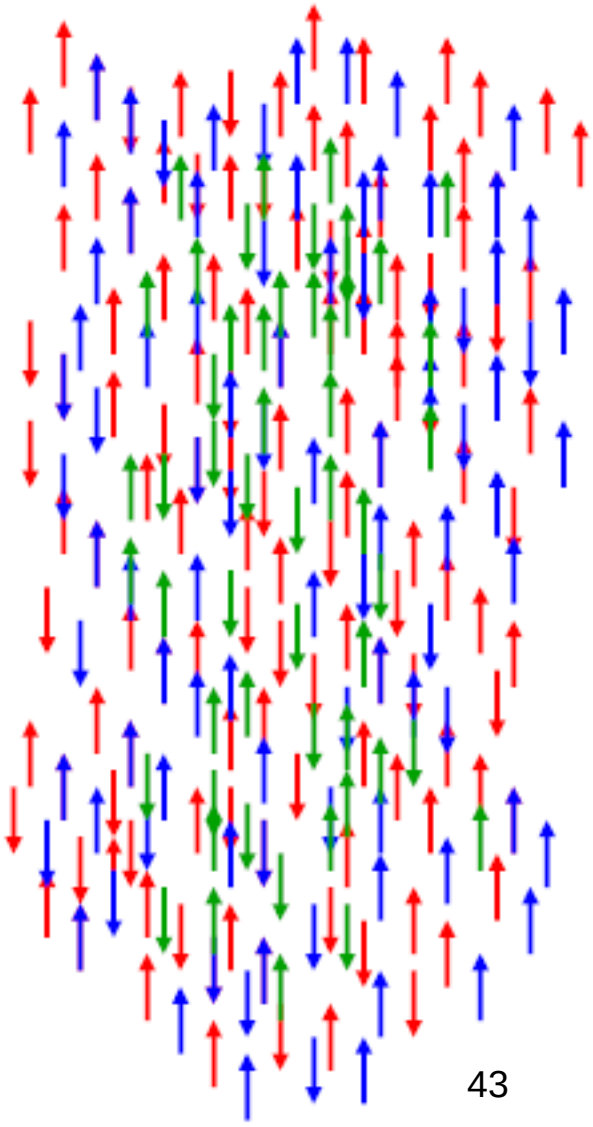
^{1}H mezi sebou, ale také se značenými ^{13}C a ^{15}N



Přenos magnetizace

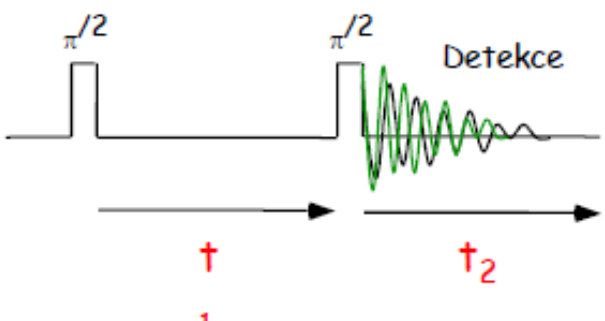
- Mezi ^{1}H, ^{13}C a ^{15}N může dojít k přenosu magnetizace, což můžeme použít k určení konektivity.



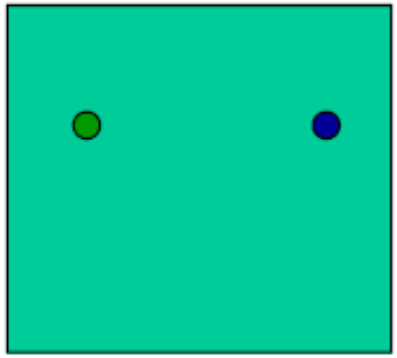


- Chemické posuny
- J-spřáhnutí (skrze vazby)
- Dipolární spřáhnutí (skrze prostor)

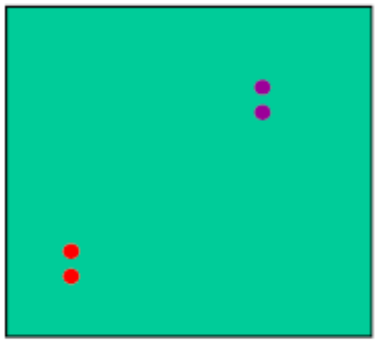
Obecný 2D experiment



FT

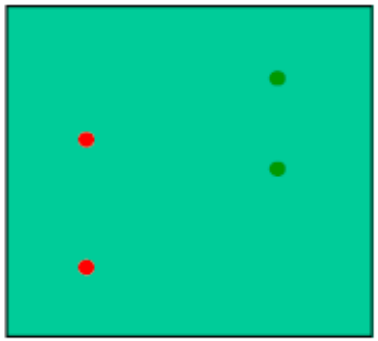


f1



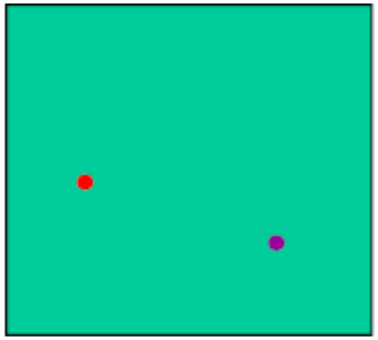
J spřáhnutí

¹H chemický posun



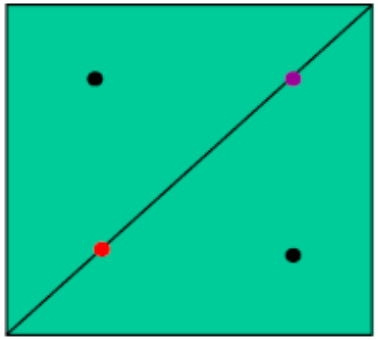
Dipolární spřáhnutí

¹H chemický posun



¹³C chemický posun

¹H chemický posun



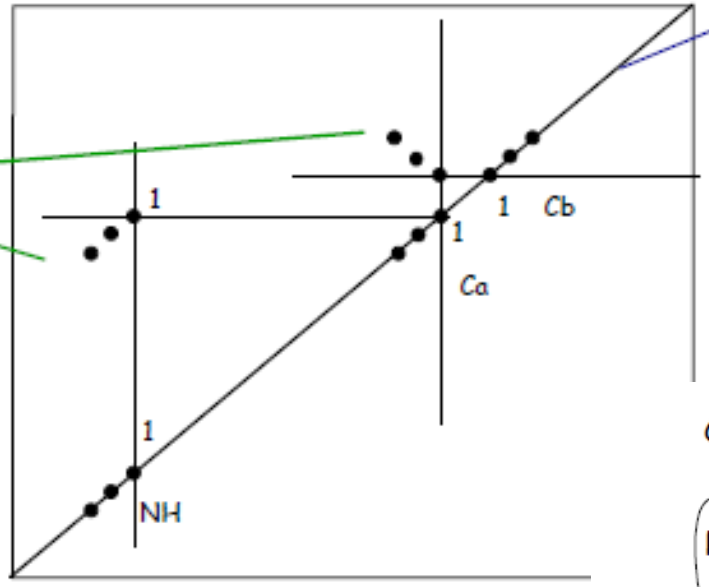
¹H chemický posun

¹H chemický posun

COSY (Correlated Spectroscopy)

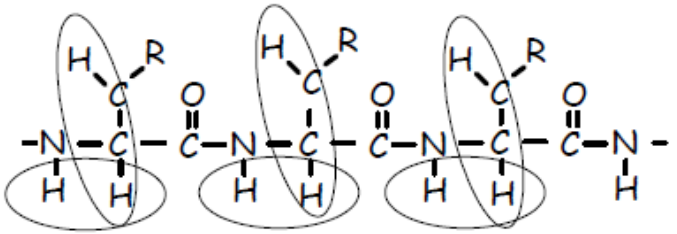
1-dimensionální spektrum je ve směru diagonály

Cross píky pocházejí z korelace skrze chemické vazby



^{1}H Chemical Shift

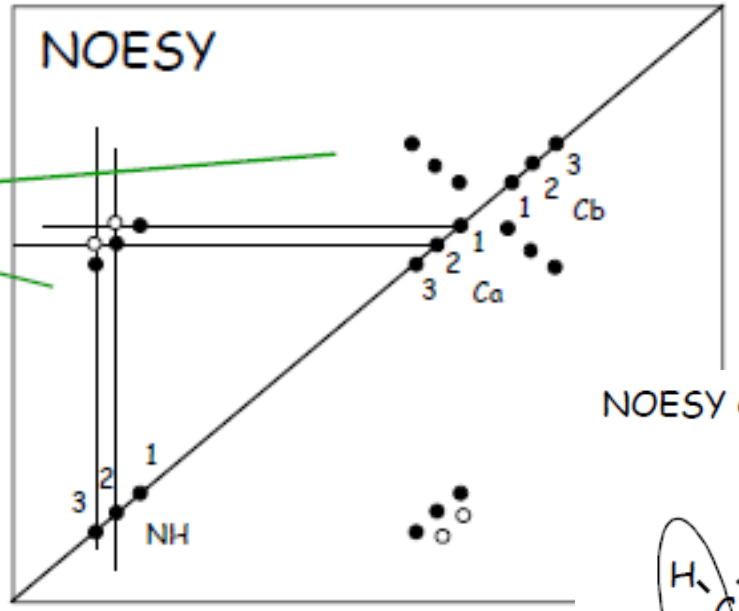
COSY Crosspeaks - ^{3}J Couplings



Cross píky jsou užitečné pro určení spinových systémů jednotlivých aminokyselin

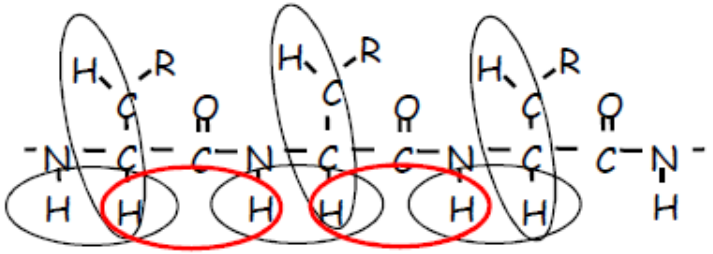
NOESY (Nuclear Overhauser Enhancement Spectroscopy)

Cross píky vznikají díky **korelaci skrze prostor**



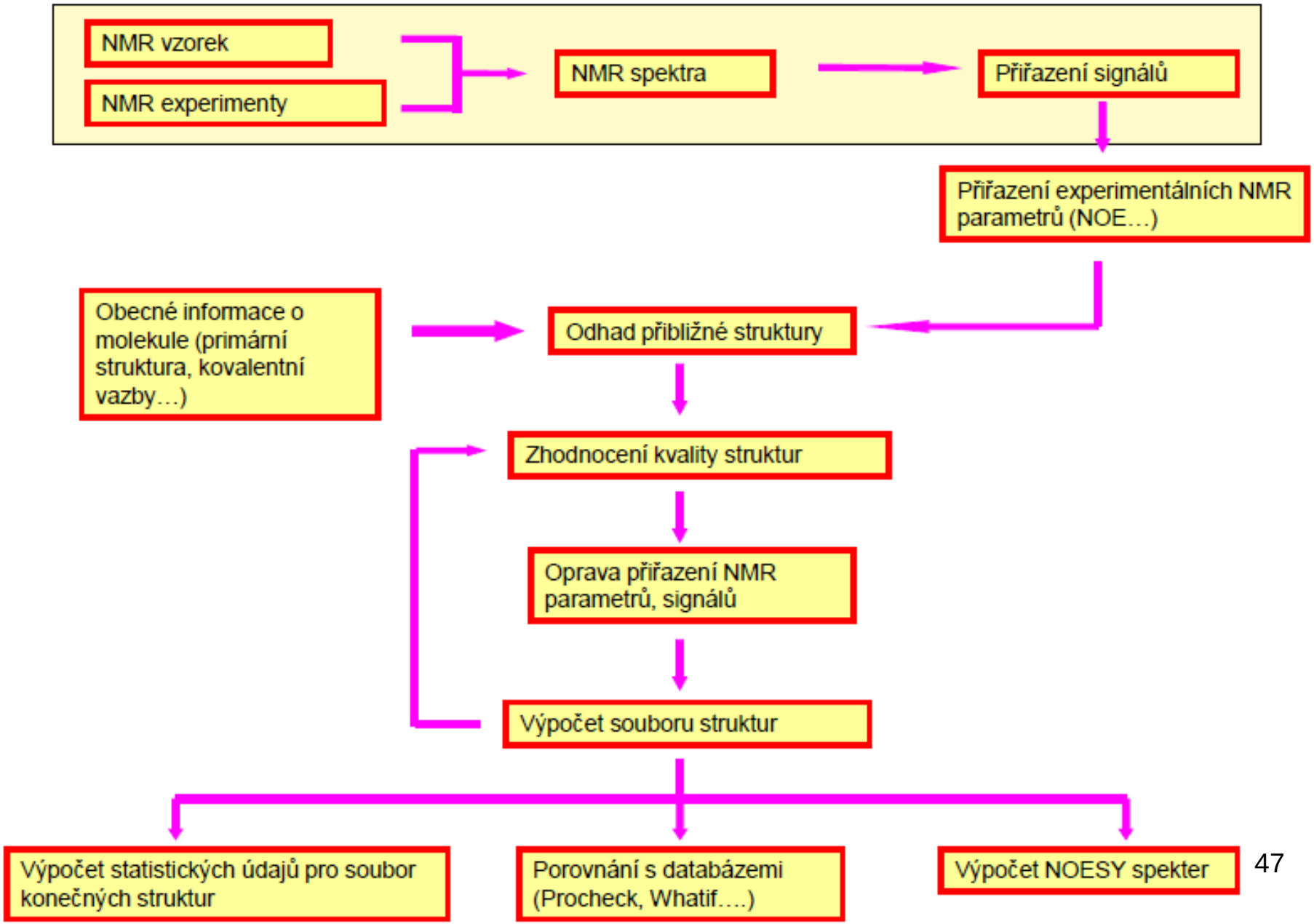
Cross píky v COSY spektru se nacházejí i v NOESY spektru

NOESY Crosspíky < 5 Å Skrze prostor



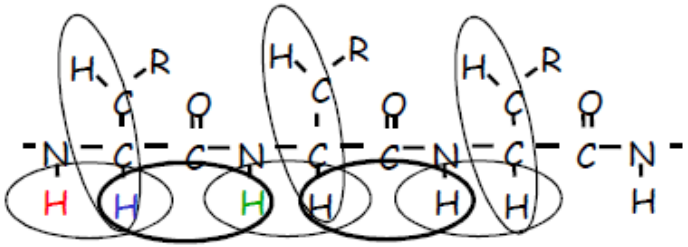
Intenzita cross-píků odpovídá inter-jaderné vzdálenosti

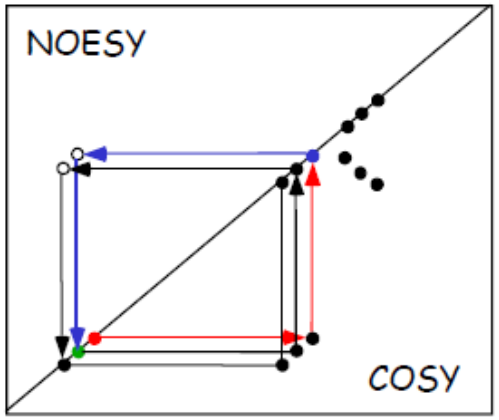
Strategie pro určování struktur biomolekul



Určení struktury pomocí NMR

- Přiřazení sekvence (určení vedlejších řetězců)
  - COSY
  - NOESY
  - 3D-NMR experimenty
- Sekundární struktura
  - chemické posuny (backbone)
  - dipolární spřáhnutí => prostor
  - J-spřáhnutí => torzní úhly
- Terciární struktura (do modelu)
  - NOE intenzity (neodpovídající COSY pro vedlejší řetězce)





Získané parametry z NMR

- Raw
  - Time-domain: 1D,2D,3D,4D spektrum
- Processed (FT)
  - frekvency domain
- NMR parametry
  - peak list
  - chemical shifts
  - 1H-1H NOE
  - J-couplings
  - residual dipolar couplings
  - NMR relaxation rates
- Derived data
  - NMR peak assignments
  - % expected in observed data
  - covalent structure
  - bond hybridizations
- Derived data
  - secondary structure
  - interatomic distances
  - torsion angles
  - hydrogen bonds
  - order parameters
  - solvent exposure
  - 3D structure
  - binding constant
  - pH titration parameters
  - hydrogen exchange rates
  - termodynamics and kinetics of structural rearangements
  - disordered regions

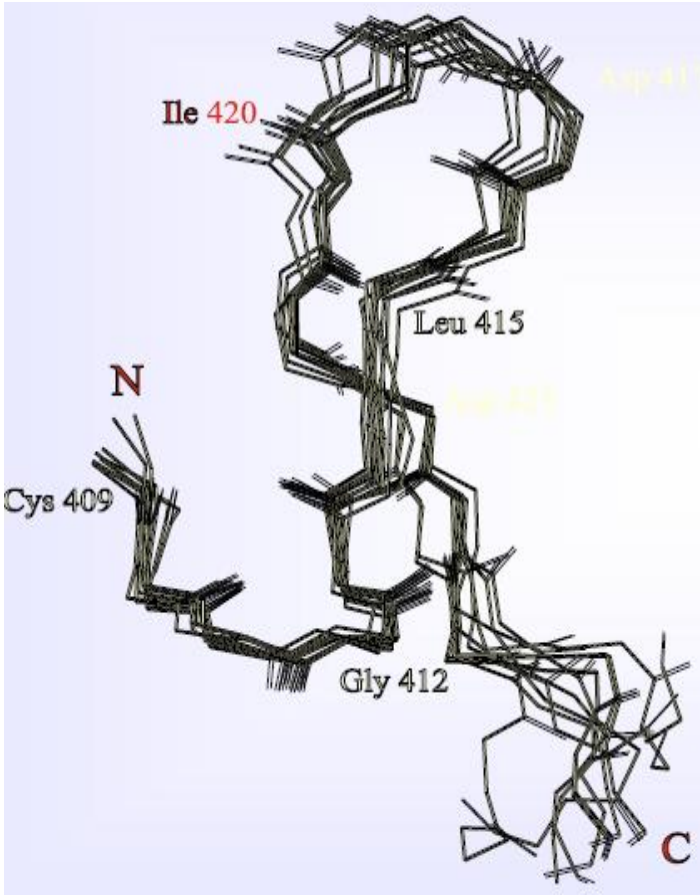
Kvalita struktury NMR

- Výsledkem NMR experimentu

= Zisk celé sady struktur, které splňují podmínky

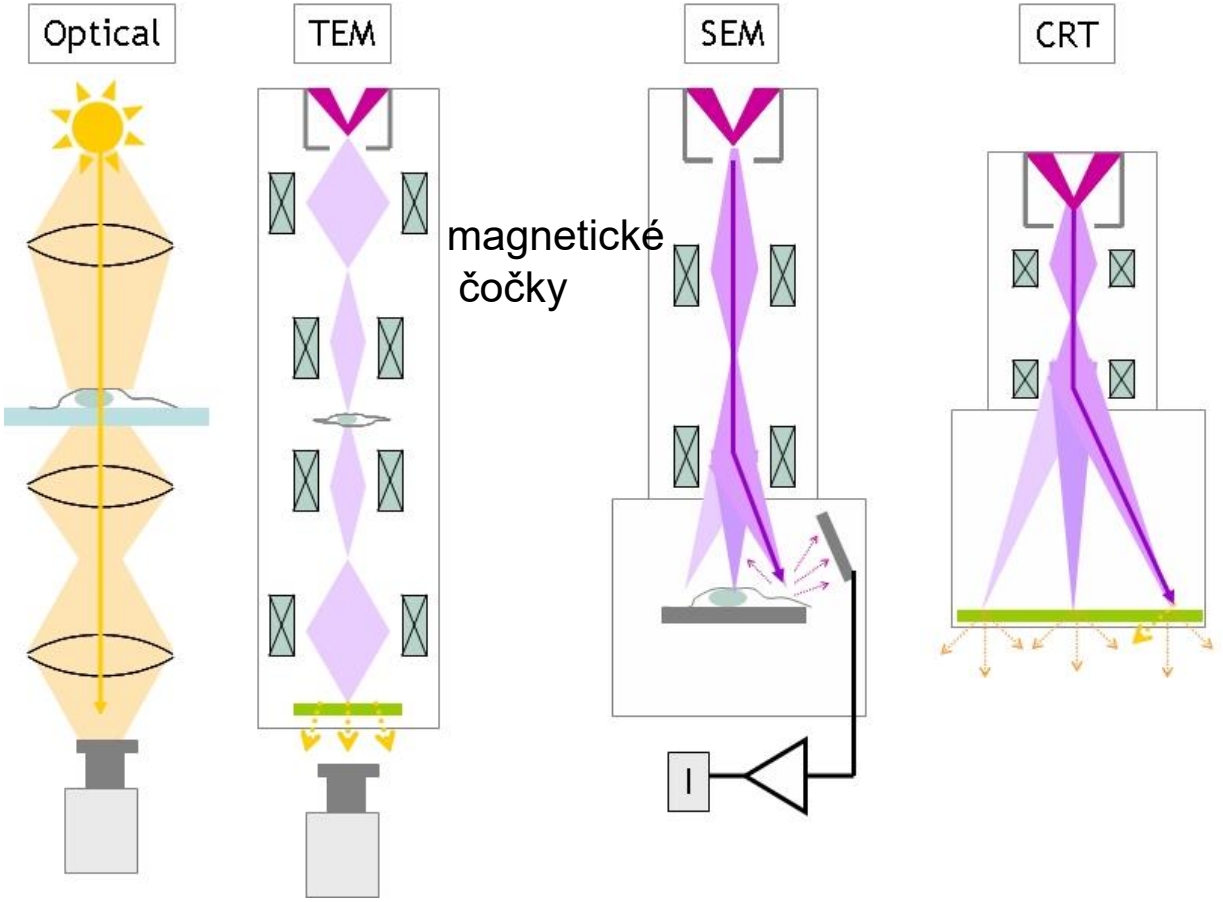
- Kvalita:
  - Stereochemie – Ramachandran
  - Ekvivalent R_{free} – vynechá se část dat při určování struktury a pak se to přes ně kontroluje

    není standardizováno!



Cryo-EM

Elektronový mikroskop – EM

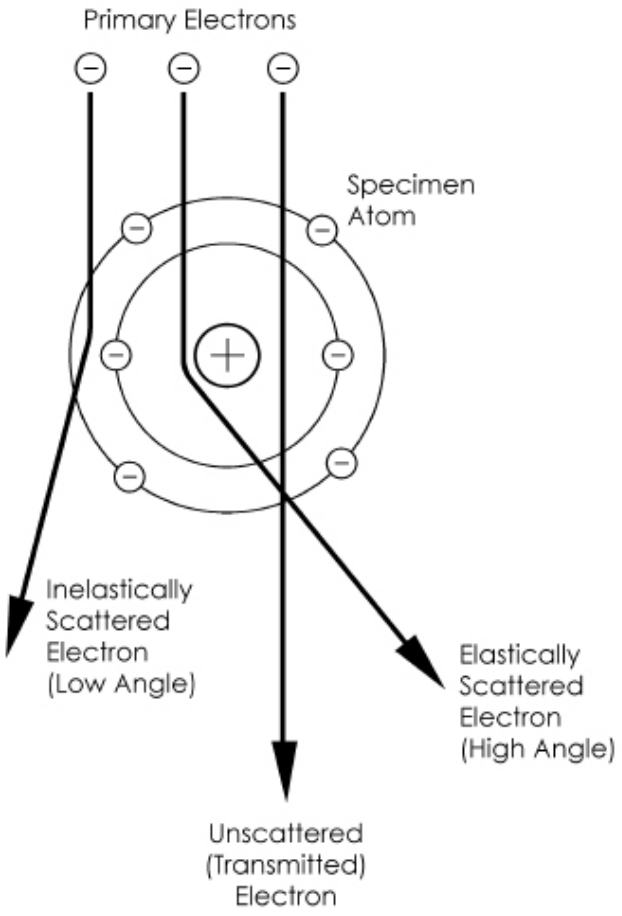


rozlišení $R = 0.61\lambda/n\sin\alpha$

- zelené světlo λ=400nm => R=150 nm
- elektrony 200 kV~ λ=0.0025nm => R=0.02nm (teoretický limit)
  R=1 nm (TEM), méně cryo-HRTEM

TEM

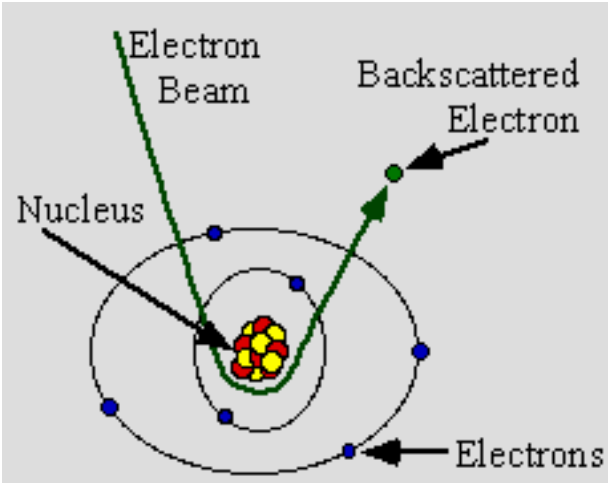
- transmisní elektronový mikroskop
- do tloušťky 100 nm

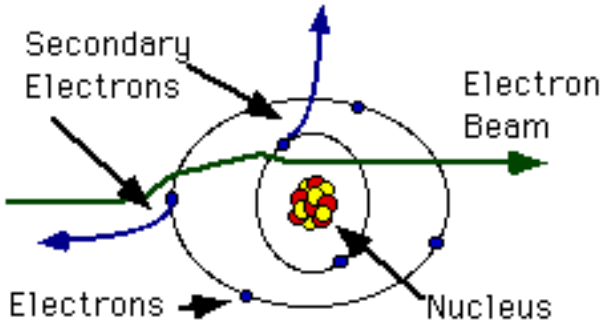


SEM

- skenovací elektronový mikroskop

- krystalografie
  - odražené e.
  - sekundární e.
  - transmitované e.
  - Xray





Elektronová tomografie

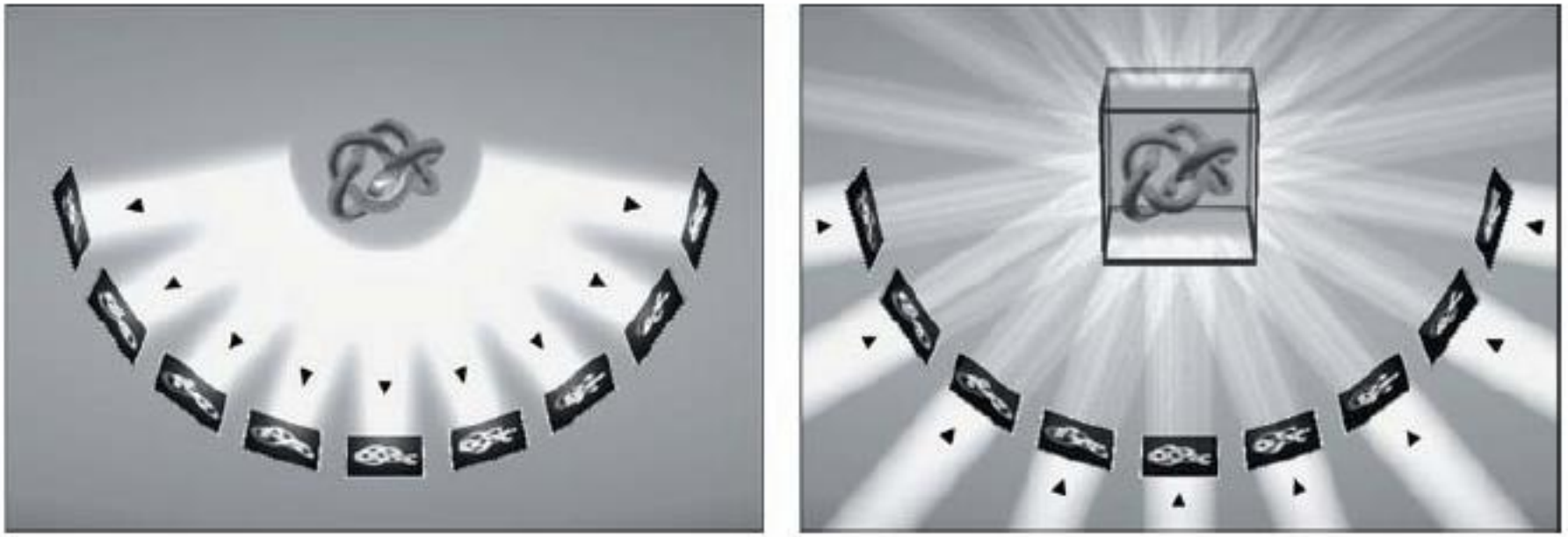
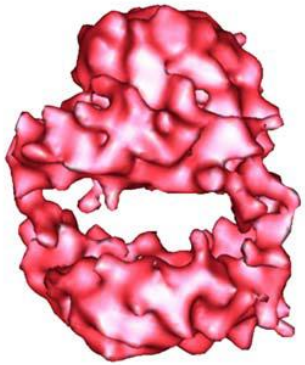


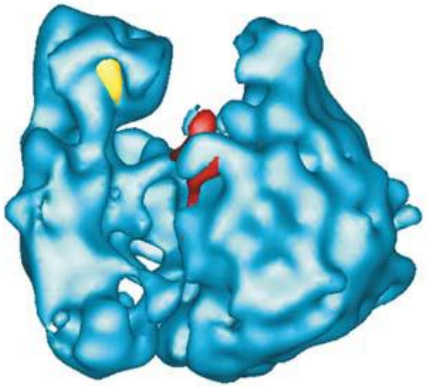
Figure 3. Electron tomography acquires multiple 2D projections of the molecule as it is tilted through a sequences of precise angular increments. The projections are then 'back-projected' to calculate the 3D model. Sidec COMET analysis acts as a mathematical filter to improve the signal to noise ratio and resolution of the model. Courtesy of Sali et al *Nature* 422:216-225; ©2003 Nature Publishing Group.

- programy: Chimera, Modeller, IMP

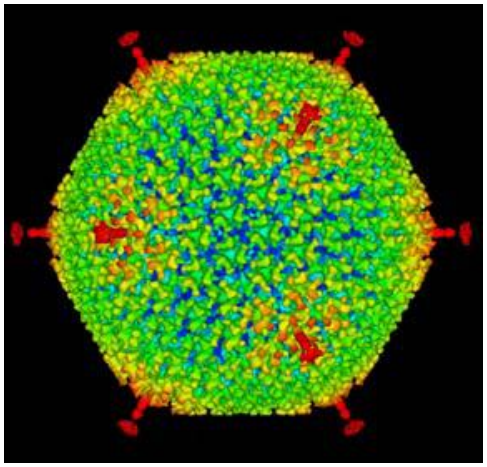
Kryoelektronová tomografie 200kDa – 400 MDa



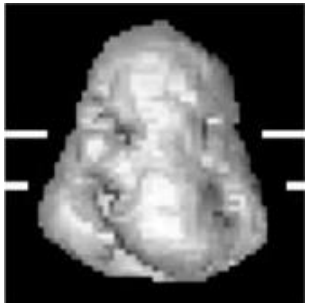
Asymmetric monomeric proteins
*e.g.*DNA-PKcs, 470 kDa

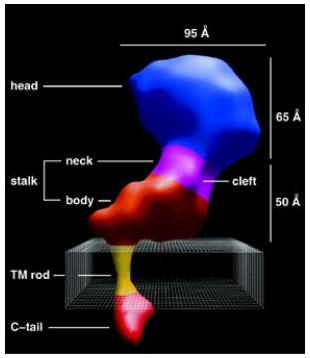


Protein/RNA or DNA complexes
*e.g.*Ribosome, ~2 MDa



Icosahedral viruses
60-fold symmetry
*e.g.*Adenovirus, ~150 MDa





Detergent solubilized membrane proteins

*e.g.*Voltage-sensitive sodium channel
~300 kDa, Sato *et al*., Nature 2001

*e.g.*Platelet integrin αIIbβ3
~230 kDa, Adair & Yeager, PNAS 2002

Cryo EM

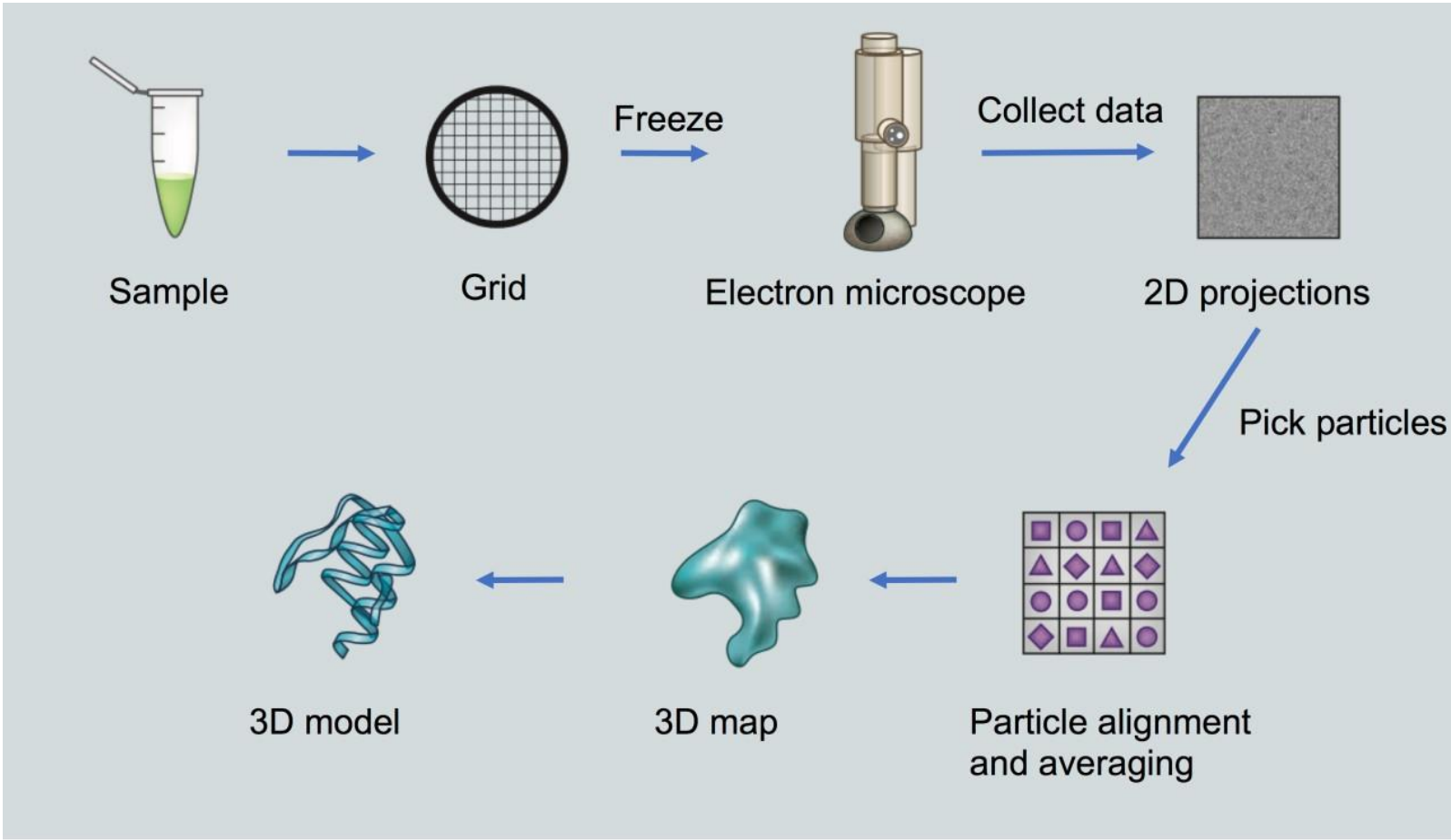
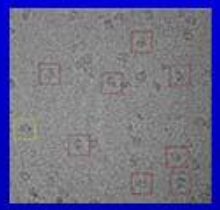
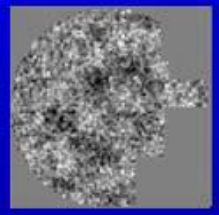

Sample
Grid
Freeze
Electron microscope
Collect data
2D projections
Pick particles
Particle alignment
and averaging
3D map
3D model

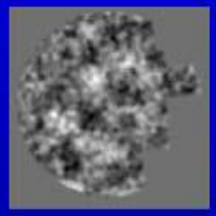
Image Processing of an Asymmetric Particle



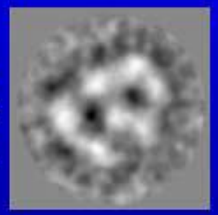
box, cut and mask particles



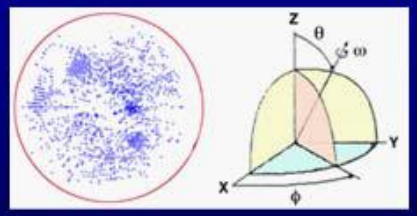
filter, invert density



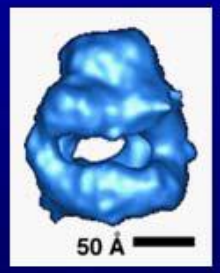
classify, average 10-15 particle images



translationally align and determine angular orientations



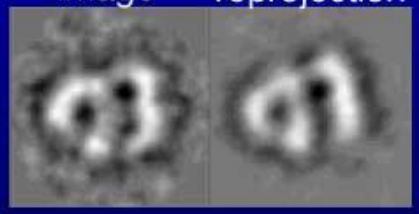
calculate 3D reconstruction



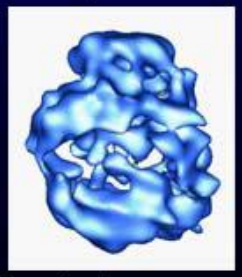
reproject

class-sum image

reprojection

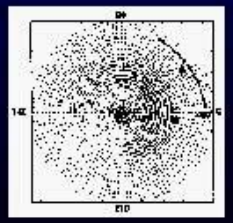


reclassify and refine orientations

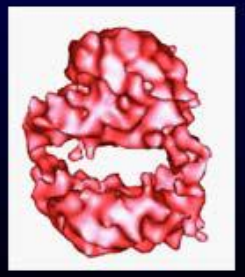


best class-sum based reconstruction

use reconstruction to determine orientations of unclassified particle images



refine particle orientations



best particle image based reconstruction

2017 NOBEL PRIZE IN CHEMISTRY

The Nobel Prize in Chemistry 2017 was awarded to Jacques Dubochet, Joachim Frank, and Richard Henderson for the development of cryo-electron microscopy for determining biomolecule structures.

X-RAY CRYSTALLOGRAPHY

Structures of proteins that form crystals

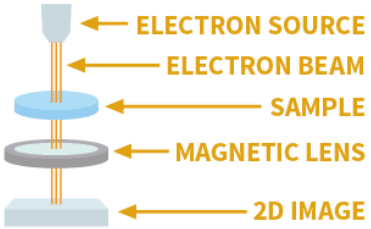
NMR SPECTROSCOPY

Structures of small proteins in solution

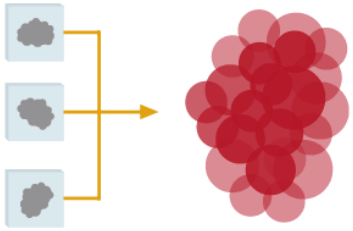
CRYO-EM

Structures of large, non-crystalline proteins

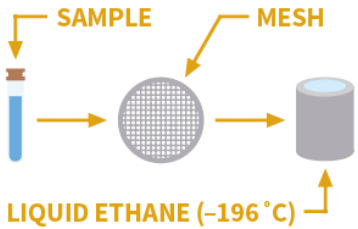
Cryo-electron microscopy (cryo-EM) is a technique that makes it possible to produce 3D images of biomolecules at atomic resolution. It can be used to capture images of biomolecules which could not be visualised with previously existing techniques.



Henderson pioneered the use of electron microscopy (EM) to visualise proteins. Using it, he produced the first atomic resolution image of a protein, bacteriorhodopsin, in 1990.



Frank developed an image analysis method that allowed computers to assemble a high resolution 3D image from many 2D EM images, improving the quality of biomolecule images.



Biological samples dry out and are damaged when in vacuum during EM. **Dubochet** solved this by rapidly freezing samples in water at –196°C to form an icy glass instead of crystals.

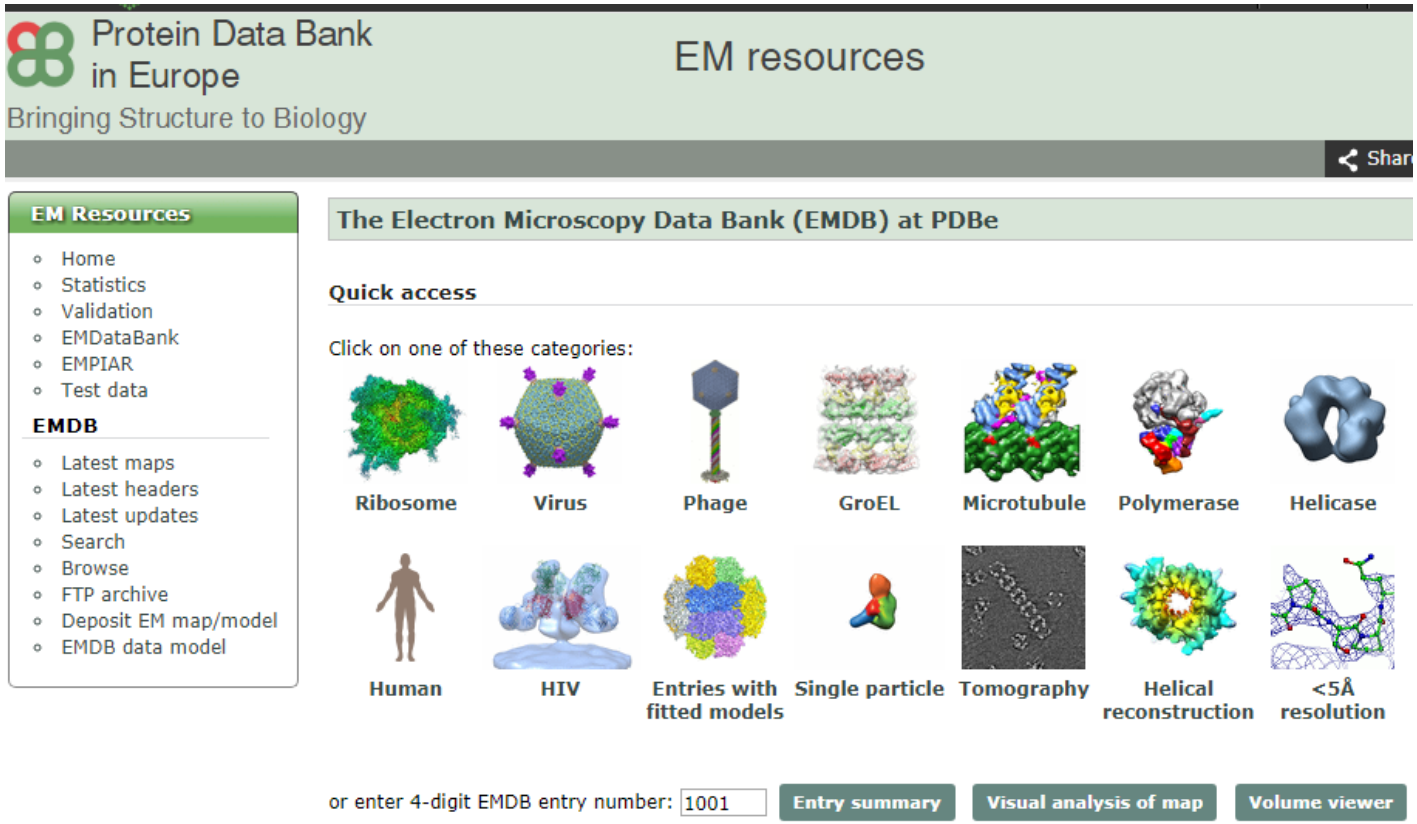
WHY DOES THIS RESEARCH MATTER?

Cryo-EM allows scientists to reveal how proteins move and interact with other molecules, freezing and observing them mid-process. It could improve our understanding of drug targets and biological processes.

Nobel Prize in Chemistry Press release: https://www.nobelprize.org/nobel_prizes/chemistry/laureates/2017/press.html

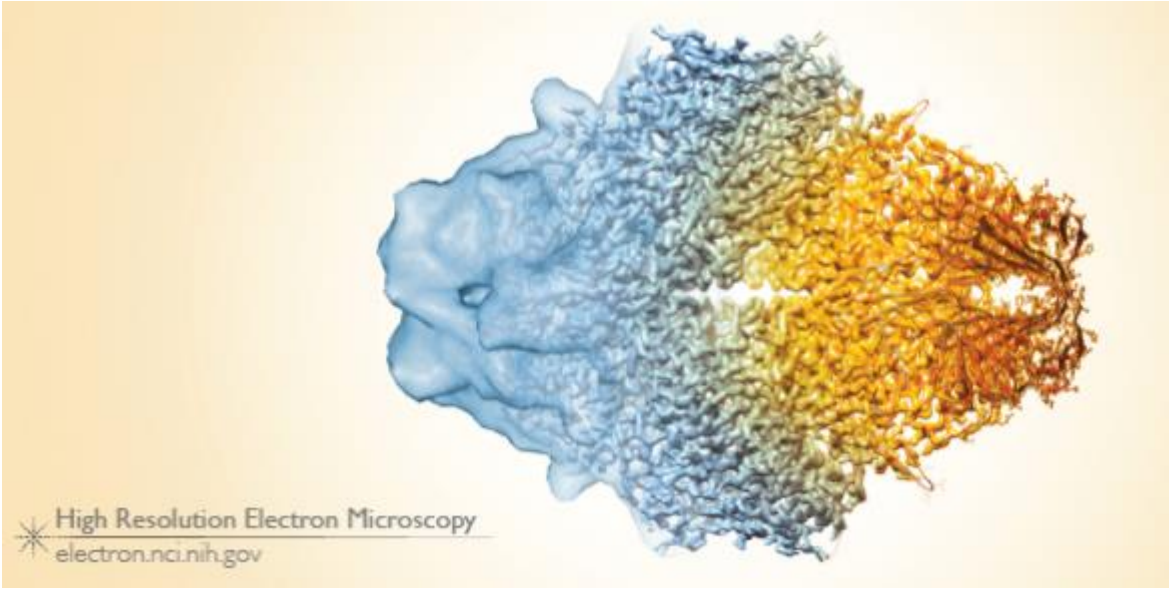
COMPOUND iNTEREST

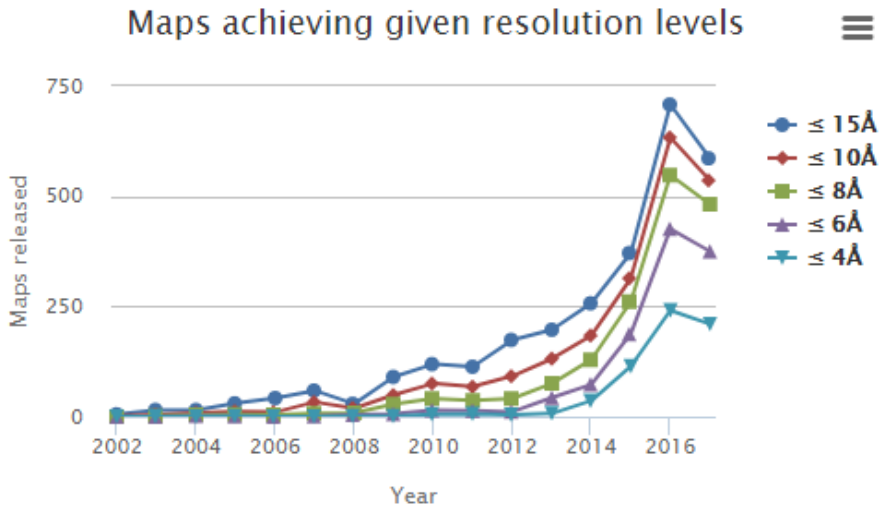
EMDB


Protein Data Bank in Europe
Bringing Structure to Biology
EM resources
EM Resources
Home
Statistics
Validation
EMDataBank
EMPIAR
Test data
EMDB
Latest maps
Latest headers
Latest updates
Search
Browse
FTP archive
Deposit EM map/model
EMDB data model
The Electron Microscopy Data Bank (EMDB) at PDBe
Quick access
Click on one of these categories:
Ribosome
Virus
Phage
GroEL
Microtubule
Polymerase
Helicase
Human
HIV
Entries with fitted models
Single particle
Tomography
Helical reconstruction
<5Å resolution
or enter 4-digit EMDB entry number: 1001
Entry summary
Visual analysis of map
Volume viewer
Introduction
The Electron Microscopy Data Bank (EMDB) is a public repository for electron microscopy density maps of macromo
and subcellular structures. It covers a variety of techniques, including single-particle analysis, electron tomogra
(2D) crystallography.
The EMDB was founded at EBI in 2002, under the leadership of Kim Henrick. Since 2007 it has been operated joi
and the Research Collaboratory for Structural Bioinformatics (RCSB PDB) as a part of EMDataBank which is fund
grant to PDBe, the RCSB and the National Center for Macromolecular Imaging (NCMI).

EMDB

- V poslední době výrazné vylepšení rozlišení
- teoretické limity jsou v řádu Å

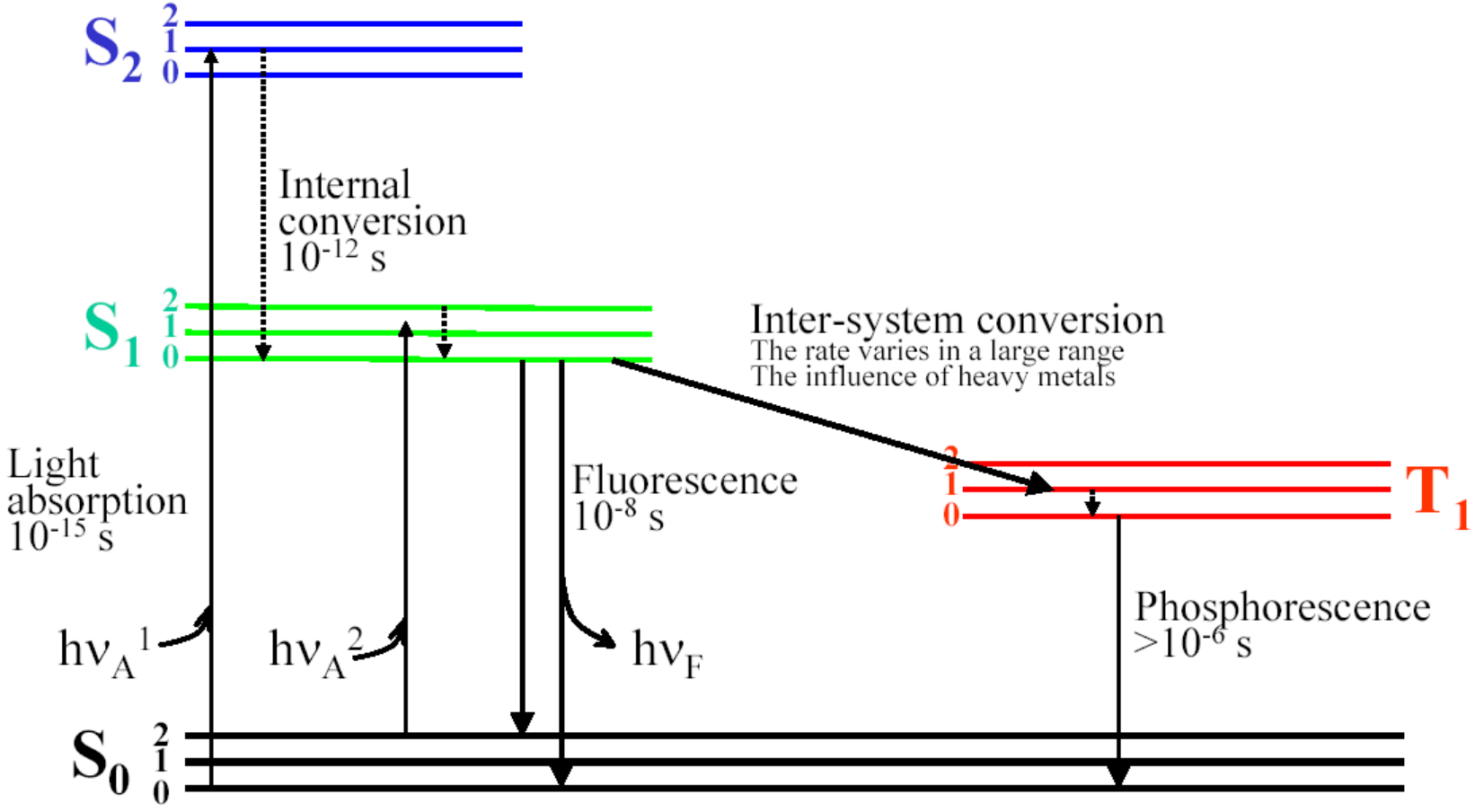




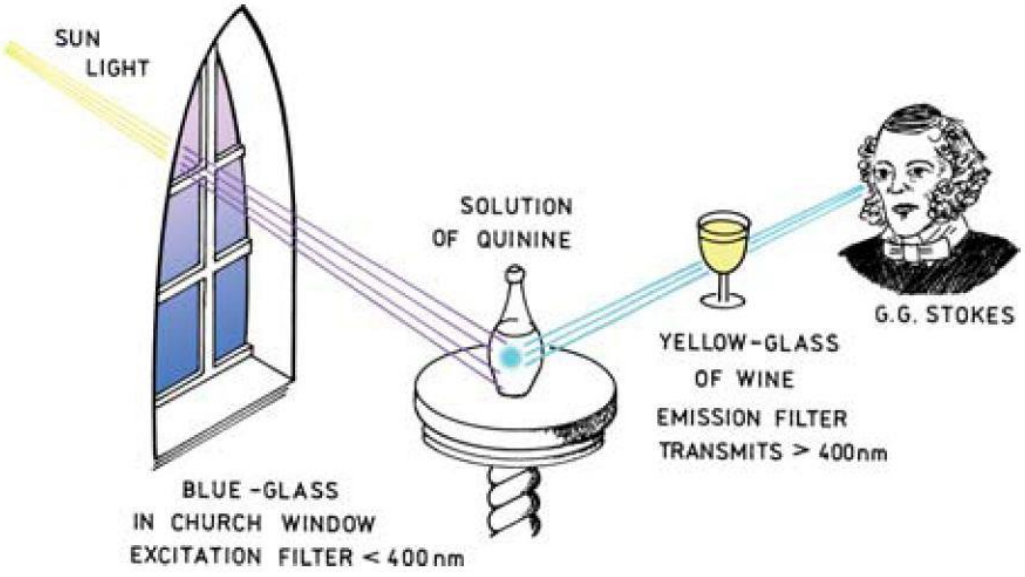
Fluorescence

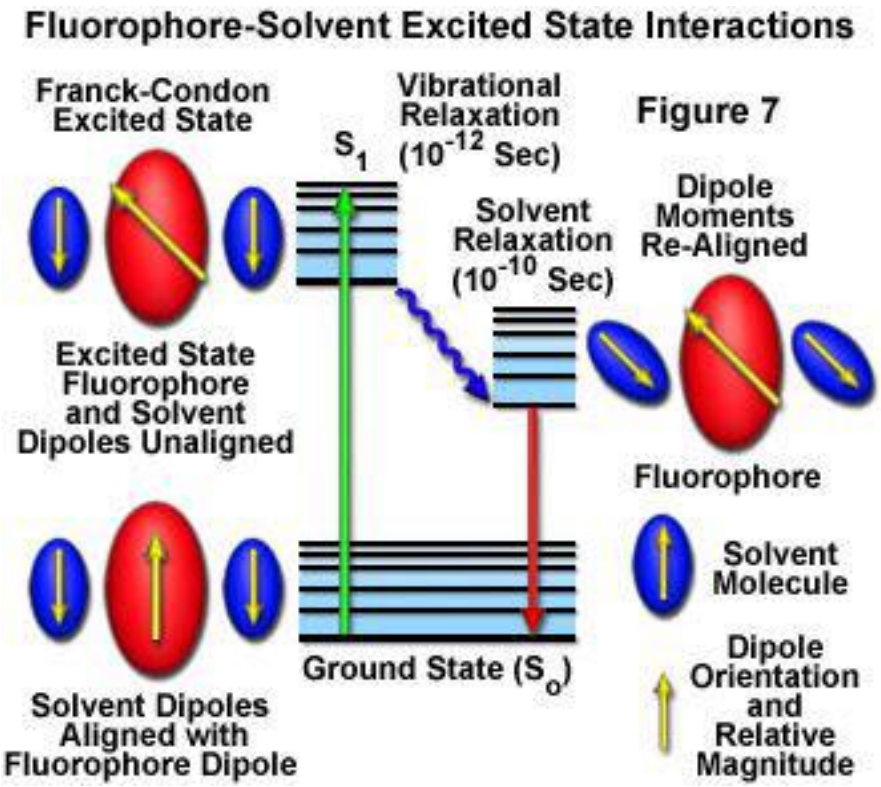
Fluorescence

- Jablonskiho diagram



Fluorescence





Z Jablonskiho diagramu vyplývá, že energie emitovaného záření (fluorescence) je typicky nižší než energie absorbovaného záření. (G.G.Stokes,1852,U.of Cambridge)

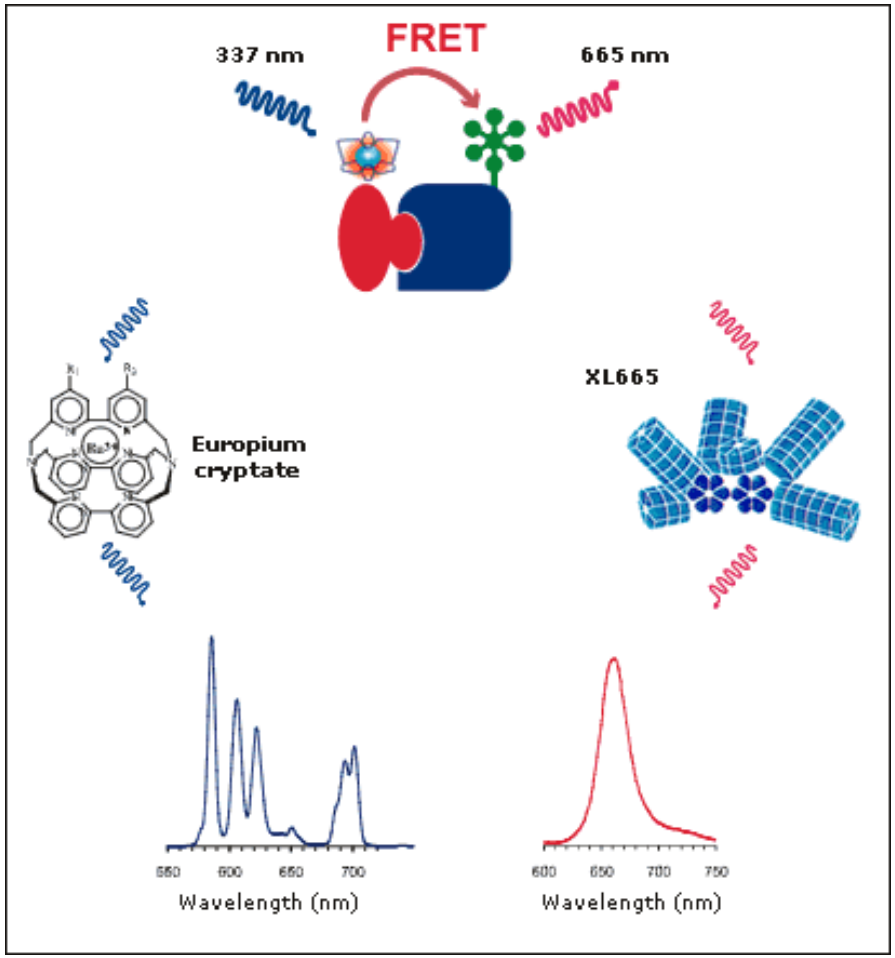
Zvýšení polarity rozpouštědla většinou způsobuje červený posun fluorescence.

Fluorescence (Förster) Resonance Energy Transfer (FRET)

- Vzdálenosti - r

$$k_{FRET}(r) = \frac{1}{\tau_0^D}\left(\frac{R_0}{r}\right)^6$$

- Když se tyto dva fluorofory dostanou blízko k sobě, tak po excitaci dojde k FRET
- R_0 je vzdálenost mezi D a A při které je přenos energie 50% (polovina energie se přenese z D na A).
- R_0 je typicky mezi 20-90Å



Typical values R_o

Donor	Acceptor	R_o (Å)
Fluorescein	Tetramethylrhodamine	55
IAEDANS	Fluorescein	46
EDANS	Dabcyl	33
Fluorescein	Fluorescein	44
BODIPY FL	BODIPY FL	57
Fluorescein	QSY 7,QSY 9 dyes	61

Take home message

- X-ray:
  - crystal (contacts), inner electrons – electron map
  - R do 2.5 A is ok for drug design, worse resolution -> electron envelope
  - size is not restricted, phase problem, static
- NMR
  - solution, doped proteins, atom nuclei – distances and torsional angles
  - correlation 2D, 3D-spectra, is ok for validation of homology models
  - size restriction, dynamical information possible
- EM
  - single molecules, inner electrones – electron envelopes (maps)
  - usually lacks atomic resolution (R > 5 A) – protein complexes
- Fluorescence (FRET), MS
  - distances only
  - need model

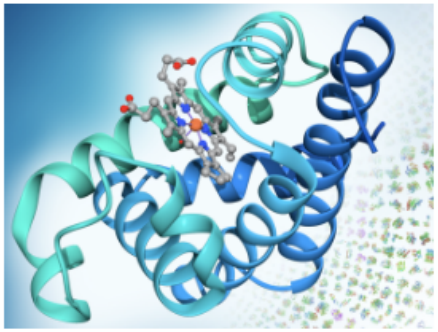
- control:
  - Ramachandran plot, R_{free} (Xray, NMR), crystal contacts, steric contacts, annotation

Nobelovské porovnání metod

X-Ray	Others
1901: Wilhelm C. Röntgen (Physics) – X-ray	1943: Otto Stern (Physics) magnetic moment of the proton (NMR)
1914: Max von Laue (Physics) diffraction of X-rays by crystals	1944: Isidor I. Rabi **(**Physics) resonance method for recording the magnetic properties of atomic nuclei (NMR)
1915: William H. Bragg and William L. Bragg (Physics) – Bragg's equation	1952: Felix Bloch, Edward M. Purcell (Physics) nuclear magnetic precision measurements (NMR)
1964: Dorothy C. Hodgkin (Chemistry) structures of penicillin and vitamin B-12.	1982: Aaron Klug (Chemistry) crystallographic electron microscopy (EM)
1985: Herbert A. Hauptman and Jerome Karle (Chemistry) **phase** problem	1986: Ernst Ruska, Gerd Binnig, Heinrich Rohrer (Physics) TEM, STM
1954: Linus Pauling (Chemistry) – chemical bond, peptide bond, and the structures of the alpha helix and beta strand	1991: Richard R. Ernst (Chemistry) high resolution nuclear magnetic resonance (NMR) spectroscopy
1962 Francis H.C. Crick, James D. Watson, Maurice H.F. Wilkins (Physiology or Medicine) – DNA	1994: Bertram N. Brockhouse and Clifford G. Shull (Physics) neutron scattering
1962: Max F. Perutz and John C. Kendrew (Chemistry) globular proteins – myoglobin, hemoglobin	2002: John B. Fenn, Koichi Tanaka (Chemistry) soft ionization mass spectrometry (MS)
1988: Johann Deisenhofer, Robert Huber, and Hartmut Michel (Chemistry) photosynthetic reaction centre (1PRC).	2002: Kurt Wüthrich (Chemistry) nuclear magnetic resonance (NMR)
1996: Paul D. Boyer, John E. Walker, and Jens C. Skou (Chemistry) F1-ATPase (1bmf, 1cow)	2003: Paul C. Lauterbur, Peter Mansfield (Physiology or Medicine) magnetic resonance imaging (MRI)
2003: Peter Agre and Roderick MacKinnon (Chemistry) membrane channels (1bl8, 2f2b, 2evu)	2013: Martin Karplus, Michael Levitt and Arieh Warshel (Chemistry) Multiscale modeling
2006: Roger Kornberg (Chemistry) molecular basis of eukaryotic transcription (1i3q, 1i50, 1i6h)	2017: Jack Dubochet, Joachim Frank, Richard Henderson (Chemistry) cryo-electron microscopy for macromolecules (CryoEM)
2009 Venkatraman Ramakrishnan, Thomas A. Steitz, and Ada E. Yonath (Chemistry) ribosome (1ffk, 1fjg, 1fka, 1gix, 1giy)	
2012 Robert J. Lefkowitz, Brian K. Kobilka (Chemistry) GPCR (3sn6, 3uon, 4daj, 4dkl)	
2020 Emmanuelle Charpentier a Jennifer Doudna (Chemistry) CRISPR-Cas9 (4cmp, 4cmq)	

Structural Biology and Nobel Prizes

The Nobel Prize highlights achievements in physics, chemistry, physiology or medicine, literature and for peace. Since its inception, many awards have recognized achievements made in molecular biology, structural biology, and related research. Molecule of the Month articles offer a starting point to explore these Prizes.



Myoglobin was the first protein visualized in 3D by X-ray crystallography, laying the foundation for a new era of biological understanding. For this discovery, John Kendrew and Max Perutz shared the 1962 Nobel Prize in Chemistry.

Year	Category	Prize	Related Molecules of the Month articles
2023	Physiology or Medicine	Katalin Karikó and Drew Weissman "for their discoveries concerning nucleoside base modifications that enabled the development of effective mRNA vaccines against COVID-19"	SARS-CoV-2 mRNA Vaccine
2022	Chemistry	Carolyn Bertozzi, Morten Meldal, K. Barry Sharpless "for the development of click chemistry and bioorthogonal chemistry"	Click Chemistry
2021	Physiology or Medicine	David Julius and Ardem Patapoutian "for their discoveries of receptors for temperature and touch"	Piezo1 Mechanosensitive Channel Capsaicin Receptor TRPV1
2020	Chemistry	Emmanuelle Charpentier and Jennifer A. Doudna "for the development of a method for genome editing"	Cascade and CRISPR
2020	Physiology or Medicine	Harvey J. Alter, Michael Houghton and Charles M. Rice "for the discovery of hepatitis C virus"	Hepatitis C Virus Protease/Helicase
2019	Physiology or Medicine	William G. Kaelin Jr., Sir Peter J. Ratcliffe, Gregg L. Semenza "for their discoveries of how cells sense and adapt to oxygen availability"	Hypoxia-Inducible Factors
2018	Chemistry	Frances H. Arnold "for the directed evolution of enzymes" George P. Smith and Sir Gregory P. Winter "for the phage display of peptides and antibodies"	Directed Evolution of Enzymes

https://pdb101.rcsb.org/learn/other-resources/structural-biology-and-nobel-prizes

Nobel Prize in Chemistry 2012

- Brian K. Kobilka
- Robert J. Lefkowitz

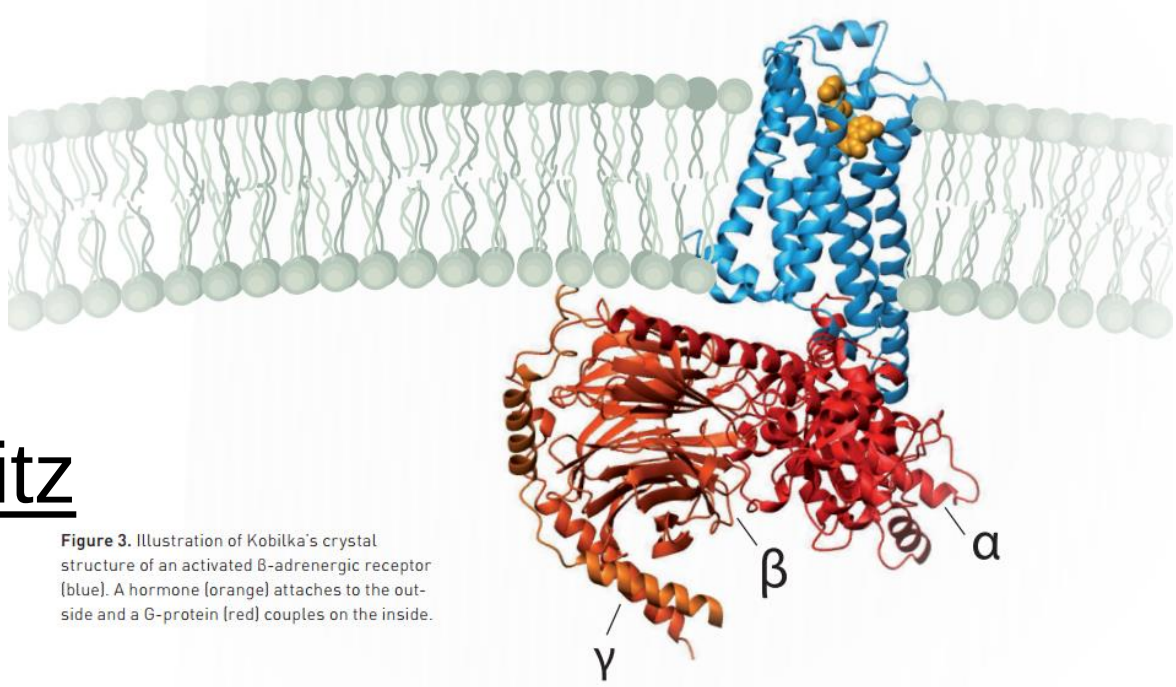


Figure 3. Illustration of Kobilka's crystal structure of an activated ß-adrenergic receptor (blue). A hormone (orange) attaches to the outside and a G-protein (red) couples on the inside.

The image, published in *Nature*, reveals new details about GPCRs, for instance, what the activated receptor looks like when it opens up a void where the G-protein likes to bind (figure 4). Such knowledge will be very useful in the future for the development of new pharmaceutical drugs.

"for studies of G-protein-coupled receptors"

Validation methods – servers

Program	Reference	Protein/NA	URL
ANOLEA	Melo and Feytmans, 1998	Protein	www.fundp.ac.be/sciences/biologie/bms
PROCHECK, PROVE, WHAT IF	EU 3-D Validation Network, 1998	Protein	ebi.ac.uk
DACA	Vriend and Sander, 1993	Protein	cmbi1.cmbi.kun.nl:1100/WIWWWI/oldqua.html
ERRAT	Colovos and Yeates, 1993	Protein	www.doe-mbi.ucla.edu/Services/ERRAT
MC-Annotate	Gendron, Lemieux, and Major, 2001	RNA	www-lbit.iro.umontreal.ca/mcannotate
MOLEMAN2	Kleywegt, 1997	Protein (Caplha)	xray.bmc.uu.se/cgi-bin/gerard/
Verify3D	Bowie, Luthy and Eisenberg, 1991	Protein	www.doe-mbi.ucla.edu/Services/Verify 3D

Validation methods – programs

Program	Reference	URL
ADIT	PDB	pdb.rutgers.edu/software
ERRAT	Colovos and Yeates, 1993	www.doe-mbi.ucla.edu/People/Yeates/Gallery/Errat.html
PROCHECK	Laskowski et al., 1993	www.biochem.ucl.ac.uk/~roman/procheck/procheck.html
PROVE	Pontius, Richelle, and Wodak, 1996	www.ucmb.ulb.ac.be/SCMBB/PROVE
SQUID	Oldfield, 1992	www.yorvic.york.ac.uk/~oldfield/squidmain.html
WHATCHECK	Hooft et al., 1996	www.cmbi.kun.nl/gv/whatcheck
WHAT IF	Vriend, 1990	www.cmbi.kun.nl/whatif